The effects of resistance training on motor unit firing rates and

recruitment during submaximal contractions

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ABSTRACT

Introduction: Previous investigations regarding the effects of resistance training on motor unit (MU) behavior during submaximal contractions have yielded inconclusive results. These investigations have predominantly analyzed composite firing rate averages, neglecting the recruitment thresholds and sizes. Analysis of MU firing rates in relation to recruitment threshold and size could provide additional insight in changes in MU recruitment patterns, which could influence reported firing rates. Though resistance training may increase fatigue resistance and twitch potentiation, the influence of such adaptations on MU behavior during submaximal contractions is unknown. **Methods:** We analyzed the influence of resistance training on MU behavior via cross-sectional comparisons of highly resistance trained individuals and physically active controls and longitudinal analyses before and after an eight-week resistance training intervention. The first project analyzed the relationships between MU firing rates, sizes and recruitment threshold in the highly resistance trained individuals and sedentary controls to determine if long-term training altered MU behavior during submaximal contractions. The second project analyzed the relationships between MU firing rates, sizes and recruitment threshold before and after the resistance training intervention to determine if the intervention altered MU firing rates and recruitment during submaximal contractions. The third project analyzed MU behavior during repetitive contractions before and after the resistance-training intervention to determine if training induced increases in potentiation could further reduce the muscle activation required to sustain a given force. **Conclusions:** Highly resistance trained individuals demonstrated reduced MU firing rates and recruitment during 40% MVC contractions compared to the physically active controls. The resistance training intervention

elicited significant MU hypertrophy altering the relationship between MU firing rate and size. In addition, the resistance training intervention reduced the muscle activation required to produce the same absolute force. Data from the repetitive contractions indicated that subjects were able to perform the second post-training contraction with reduced MU recruitment relative to the first post-training contraction, likely, as a result of resistance training induced increases in twitch potentiation. Together the projects demonstrate that resistance training can alter MU firing rates and recruitment during submaximal contractions. These adaptations reduce the muscle activation required to sustain a given force, thus reducing fatigue.

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1. INTRODUCTION

1.1 BACKGROUND

Resistance training is well documented to increase strength and induce muscle hypertrophy (1). Though muscle hypertrophy can contribute to observed strength gains, several studies have reported strength increases absent significant hypertrophy (2-4). In addition, an increase in muscle activation during maximal contractions, as measured by electromyography (EMG), is a commonly observed adaptation to resistance training (3, 5, 6). The combination of these two factors has led researchers to conclude that early strength gains are largely due to neuromuscular adaptations.

The force produced by a muscle is a factor of both the number of motor units (MUs) recruited and the firing rate of the active MUs. Thus, researchers have examined the effects of resistance training on MU recruitment and firing rates during maximal and submaximal contractions. Increases in voluntary activation have been reported following resistance training, however, the increases were small as muscle activation was already high in the measured muscles (7, 8). As such, increases in voluntary activation are unlikely to fully explain the large increases in strength and EMG amplitude observed post resistance training.

Additional investigations have analyzed the effects of resistance training on MU firing rates during maximal and submaximal contractions with mixed results. Previous investigations have reported increases (9-11) and no changes (8) in maximal firing rates following resistance training investigations, though the reported maximal firing increases in several investigations are questionable. Similarly, increases and no change in firing rates during submaximal contractions have also been reported (8, 9, 12-15). The majority of these investigations have analyzed firing rates without accounting for recruitment thresholds, which could significantly influence the

reported average firing rates (7, 8, 13, 15). Even those studies that did account for recruitment threshold, did not examine the sizes of the active MUs (12, 14). Data regarding the size of active MUs could provide additional insight regarding resistance training induced alterations in MU recruitment, and determine if resistance training alters the relationship between MU firing rates and MU size.

Lastly, though fatigue (16-21) and twitch potentiation (18, 22-24) are known to influence muscle activation during repeated contractions, the influence of resistance training on MU firing rates and recruitment during repetitive contractions is not well understood.

Therefore, the aim of these investigations is to provide a better understanding of resistance training induced neuromuscular adaptations at the MU level. Specifically, we aim to determine how long and short-term resistance training influence MU firing rates, sizes and recruitment during submaximal contractions. Additionally, we aim to determine if resistance training induced increases in fatigue resistance and twitch potentiation can alter MU firing rates and recruitment during repetitive submaximal contractions.

1.2 SPECIFIC AIMS

This proposal consists of three projects from two separate studies. The first study consists of a cross-sectional comparison of highly-resistance trained individuals and physically active controls. MU firing rates, sizes and recruitment thresholds during submaximal contractions of the first dorsal interosseous were analyzed to determine the influence of long-term resistance training on MU behavior. The second study consists of a longitudinal investigation of changes in MU behavior before and after an eight-week resistance training intervention. The second project will analyze MU firing rates, sizes and recruitment thresholds during submaximal contractions of

the vastus lateralis to determine how short-term resistance training affects MU firing rates, sizes and recruitment during submaximal contractions at the same absolute and relative intensity as pre-training. Additionally, changes in peak torque and muscle cross-sectional area will be examined. The third project will examine whether the resistance training intervention altered MU firing rates and recruitment during repetitive contractions as a result of increase fatigue resistance or MU twitch potentiation

1.2.1 Study 1: Differences in the motor unit firing rates and amplitudes in relation to recruitment thresholds during submaximal contractions of the first dorsal interosseous between the chronically resistance trained and physically active men

Specific Aim 1: Determine if highly resistance trained demonstrated greater peak index finger abduction force and first dorsal interosseous cross-sectional area than physically active controls.

Hypotheses: We hypothesized that the highly trained individuals would demonstrate significantly greater force and muscle cross-sectional area.

Specific Aim 2: Determine if MU firing rates and recruitment differed between highly resistance trained individuals and physically active controls.

Hypotheses: We hypothesized that the highly trained individuals would demonstrate reduced MU firing rates recruitment at 40% but not 70% MVC in comparison the physically active controls

1.2.2 Resistance training increases motor unit sizes, but does not alter firing rates

Specific Aim 1: Determine if the resistance training intervention increased strength and cross-sectional area of the vastus lateralis.

Hypotheses: We hypothesize that the training intervention will significantly increase isometric knee-extension torque and the cross-sectional area of the vastus lateralis.

Specific Aim 2: Determine if the resistance training intervention altered firing rates and recruitment during contractions at the same absolute intensity as pre-training.

Hypotheses: We hypothesized that the resistance training protocol would reduce MU recruitment during contractions at the same absolute force as pre-training. Alternatively, we hypothesized that minimal changes in firing rates would be observed.

Specific Aim 3: Determine if the resistance training intervention induced MU specific hypertrophy

Hypotheses: We hypothesized that the resistance training protocol would alter the relationship between $MUAP_{AMP}$ and recruitment threshold indicating hypertrophy of the high-threshold MUs.

Specific Aim 4: Determine if the resistance training protocol altered MU firing rates and recruitment during contractions at the same relative intensity as pre-training.

Hypotheses: We hypothesized that resistance training would not alter MU recruitment at the same relative intensity. In regards to firing rates, we hypothesized that resistance training would not alter firing rates in relation to recruitment threshold. As a result of the significant MU hypertrophy, we hypothesized that firing rates in relation to MUAPAMP would be significantly altered following the resistance training intervention.

1.2.3 The effects of resistance training on motor unit firing rates and recruitment during repetitive submaximal contractions

Specific Aim 1: Determine if resistance training reduced the excitation required to produce the same absolute force.

Hypotheses: We hypothesized that as a result of the resistance training induced strength gains, subjects would be able to perform both post-training contractions with reduced excitation to the motoneuron pool.

Specific Aim 2: Determine if resistance training induced increases in twitch potentiation could reduce the firing rates and recruitment required to perform the second post-training contraction relative to the first post-training contraction.

Hypotheses: We hypothesized that training would increase MU twitch force potentiation, thus reducing the excitation required to perform the second contraction. As a result of the reduced excitation, firing rates and recruitment would be reduced relative to the first post-training contraction.

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2. REVIEW OF LITERATURE

2.1 MOTOR UNIT RECRUITMENT

The Size Principle

Motor units (MUs) are recruited in an orderly manner according to the size of their alpha motoneurons as first reported by Henneman (1). In this investigation, Henneman applied an electrical stimulus to the dorsal roots of decerebrated cats to elicit reflexes in the lumbar ventral roots. Progressively larger electrical stimuli were delivered to the dorsal roots or large nerve trunks and the elicited impulses in the ventral root filaments were recorded. Larger impulses were associated with larger motoneurons. As the intensity of electrical stimulation increased, progressively larger impulses were observed, indicating the recruitment of larger motoneurons. Henneman concluded that the susceptibility of a motoneuron to discharge is related to its size, thus motoneurons are activated in order of increasing size. In a follow-up examination of the relationship between motoneuron size and excitability, Henneman et al concluded that the greater excitability of the small motoneurons was likely due to the input resistance; the greater input resistance of smaller motoneurons contributed to their increased excitability (2). In contrast, the smaller input resistance of larger motoneurons contributed to their decreased excitability. In this later investigation, Henneman first referred to the orderly recruitment of MUs according to their motoneuron size as the "size principle"

Following the early work by Henneman, numerous researchers have corroborated the size principle in humans. Orderly recruitment of MUs according to size have been reported in concentric and eccentric dynamic contractions (3-5), as well as during isometric contractions (3, 6-11). Furthermore, the size principle has been observed in numerous muscles, including the

biceps brachii (4, 5), flexor carpi radialis (3), first dorsal interosseous (6-8, 10), masseter (11) and vastus lateralis (9). Though individual MU recruitment thresholds can be increased or decreased, the size principle is unaltered by aging (7, 10), fatigue (12, 13) or exercise training (9). Of note two investigations have reported a lack of orderly recruitment in MUs with recruitment thresholds below 10% MVC. Westad and Westgaard reported similar MU sizes in the trapezius during isometric contractions at 10% MVC (14). Similarly, Sogaard observed a lack of orderly recruitment in biceps brachii MUs recruited during 10% MVC dynamic and isometric elbow flexion and extension contractions. In both investigations, researchers concluded that the results did not contradict the size principle, but suggested that MUs recruited before 10% MVC were of similar size due to similar motorneuron sizes and muscle fiber compositions.

Analysis of motor unit size

To measure MU size in vivo in human subjects, researchers have analyzed the amplitude of the MU action potentials ($MUAP_{AMP}$) due to its correlation with the size of the motoneuron and the diameter of the MU's component muscle fibers. Gasser reported a direct relationship between the diameter of nerve fiber and the amplitude of nerve impulses recorded from peripheral nerves (15). Later, Olson et al(16) employed nerve stimulation in decrebrated cats to progressively recruit larger MUs with larger motoneurons. As the size of the MU increased, so did the peak to peak amplitude of the $MUAP_{AMPS}$ measured in the posterior biceps and semitendinosus, thus establishing the relationship between motoneuron size and $MUAP_{AMP}$. Hakansson (17) delivered electrical stimuli and measured the amplitude of the resulting action potentials in isolated frog muscle fibers to establish the relationship between muscle fiber

diameter and action potential amplitude. Together, these seminal investigations form the basis of the assumption that a MU's size can be estimated via the analysis of the amplitude of its action potential.

Analysis of $MUAP_{AMPS}$ in humans has traditionally been performed via spike trigger averaging, a methodology established by Milner et al (6), which employs both surface and intramuscular electrodes. The intramuscular spikes corresponding to motoneuron firings serve as triggers around which the surface EMG signal for a given epoch can be averaged to estimate the peak-to-peak $MUAP_{AMP}$. Using this methodology, Milner et al observed a strong relationship between $MUAP_{AMP}$ and recruitment threshold in agreement with the size principle.

Recent advances in EMG technology allows for the decomposition of surface EMG signals into the action potential trains of individual MUs (18-20). This method employs a surface sensor array that collects four channels of EMG data, from which a decomposition algorithm yields the action potential trains and firing instances of individual MUs. Additionally, the algorithm yields a representative action potential waveform from each of the four channels. The $MUAP_{AMP}$ can be calculated by averaging the peak-to-peak amplitude of the four waveforms (8). Using surface EMG decomposition, researchers have similarly observed strong positive relationships between $MUAP_{AMP}$ and recruitment threshold (7-9).

Though the size principle is widely accepted, there is some debate as to the validity of $MUAP_{AMPS}$ measured via surface EMG. A recent investigation by Del Vecchio (21) reported weak correlations between recruitment threshold and MUAP EMGRMS, however, there are important differences in the calculation of $MUAP_{AMP}$ using the aforementioned decomposition algorithm and MUAP EMGRMS. With the decomposition algorithm, the representative MUAP

waveforms are first identified and then used to identify the firing instances of the individual MUs. In contrast, Del Vecchio et al first identified the firing instances of the MUs and then calculated the average EMGRMS in the epoch surrounding the firing instance. Additionally, the weak MUAP EMGRMS vs. recruitment threshold relationships reported by Del Vecchio were constructed using composite data from multiple contractions at different intensities, whereas investigations reporting strong $MUAP_{AMP}$ vs. recruitment threshold relationships have used MU data from single contractions (7-9). Interestingly, a more recent publication from the same research group reported significant composite MUAP EMG RMS vs. recruitment threshold relationships following an EMG normalization procedure (22).

Analysis of MU size adaptations via the slope of the $MUAP_{AMP}$ vs. recruitment threshold relationship

In addition to demonstrating the size principle, $MUAP_{AMP}$ can be expressed relative to recruitment threshold to analyze the threshold specific adaptations in MU size such as MU hypertrophy and atrophy. In these investigations, $MUAP_{AMPs}$ have been linearly regressed against recruitment thresholds to yield a slope and y-intercept value for each individual contraction. The mean slopes and y-intercepts can then be statistically analyzed. A change in the slope would indicate a non-uniform change in MU size along the recruitment threshold spectrum, whereas a change in y-intercept value would indicate a uniform change.

Pope et al analyzed $MUAP_{AMP}$ vs. recruitment threshold relationships from vastus lateralis MUs to determine the effect of resistance training on MU sizes. The parameters of the MUAPAMP vs. recruitment threshold relationships of vastus lateralis MUs were analyzed before

and after an eight-week resistance training intervention. Three times per week, subjects performed three sets of ten repetitions for seven exercises, three of which targeted the knee extensors. In addition to the MU analyses, changes in vastus lateralis muscle cross-sectional area were analyzed via ultrasound imaging. Pre-training, the composite MU data demonstrated a linear relationship between $MUAP_{AMP}$ and recruitment threshold, where as post-training the relationship was best fit by an exponential model. Pope et al attribute this change in the relationship to negligible changes in the low threshold MU sizes, but progressively larger increases in MU size along the recruitment threshold beginning at recruitment thresholds of approximately 30% MVC. Analysis of individual relationships demonstrated a 31.7% increase in slope for the full relationship. Separate relationships were also analyze for low (<30% MVC) and high threshold MUs (30% MVC). No change in relationship slopes were observed for the low-threshold MUs, but a significant increase in slope was observed for the high-threshold MUs. Furthermore, the increase in the full relationship slopes was highly correlated ($r^2 = 0.836$) with increases in vastus lateralis CSA. Thus, analysis of MUAPAMP vs. recruitment threshold slopes provides a non-invasive measure of recruitment threshold specific MU hypertrophy.

In a cross-sectional design, Sterczala et al (7) compared $MUAP_{AMP}$ vs. recruitment threshold slopes of the first dorsal interosseous in young and old subjects to determine the effects of sarcopenia associated muscle atrophy on MU size. In agreement with the aforementioned studies by Westad et al (14) and Sogaard et al (23), neglible difference in $MUAP_{AMPS}$ were observed in MUs recruited below 10% MVC and were thus excluded from the slope analyses. Older subjects (age: 61 ± 2 years) demonstrated significantly reduced slopes compared to younger subjects (22 ± 3 years). Low threshold MU sizes were similar between groups, however the difference in MU increased along the recruitment threshold spectrum such that the largest

differences in size were observed at the highest recruitment thresholds. Sterczala et al concluded that the differences in slopes indicate that aging is associated with greater atrophy in the high threshold MUs potentially due to disuse.

The association of recruitment threshold with muscle fiber type

Both Pope et al (9) and Sterczala et al (7) reported that the non-uniform changes in hypertrophy and atrophy were likely due to MUs' muscle fiber type, as the fiber type within a given MU is homogenous (24). Though a specific fiber type (e.g. type I) cannot be attributed to a given recruitment threshold or recruitment threshold range, lower and higher threshold MUs have been associated with the properties of specific muscle fiber types. Given the relationship between $MUAP_{AMP}$ and muscle fiber diameter previously discussed (17), the positive $MUAP_{AMP}$ vs. recruitment thresholds indicate that the low threshold MUs possess smaller muscle fibers than high threshold MUs, and that high threshold MU muscle fibers are more susceptible to hypertrophy with resistance training and atrophy with aging. Carpentier et al (25) reported that higher threshold MUs (25-50% MVC) in the FDI demonstrated greater fatigue during repeated contractions than lower threshold MUs (<25% MVC). An inverse relationship between recruitment threshold and twitch duration was reported by Milner-Stein et al (26). Additionally, several studies have reported a positive relationship between recruitment thresholds and twitch tension (11, 25, 26).

Perhaps the best evidence of the association between recruitment threshold and fiber type was reported by Garnett et al (27), which combined analyses of contractile properties such as contraction time and fatigue index with histochemical staining of biopsied muscle fibers. MUs

recruited at lower stimulation levels were smaller and demonstrated greater contraction times and lower twitch tensions. Additionally, constant electrical microstimulation was delivered intramuscularly to the gastrocnemius MUs for 2.5 hours at the amplitude at which various MUs were activated to glycogen deplete the muscle fibers. Subsequently, the tissue was biopsied. Biopsies in type I fibers were depleted (low Myosin ATPase, high succinate dehydrogenase) were associated with MUs demonstrating longer twitch durations and lower twitch tensions. Biopsies in which type II fibers (high Myosin ATPase, low succinate dehydrogenase) were depleted were associated with MUs demonstrating shorter twitch durations and greater twitch tensions.

Muscle fibers demonstrate a continuous spectrum of contractile properties (28) and can coexpress multiple myosin heavy chain isoforms (29), thus it is inappropriate to oversimplify the classification of low threshold MUs as type I MUs or high threshold MUs as type II MUs. Nonetheless, previous investigations have demonstrated that properties of lower threshold MUs, greater fatigue resistance, longer twitch durations and lower twitch tensions are similar to those attributed to type I muscle fibers, whereas the properties of higher threshold MUs, reduced fatigue resistance, shorter twitch durations and higher twitch tensions are attributed to type II muscle fibers (30-33).

MU recruitment thresholds are modifiable

An important consideration, particularly as it pertains to training interventions, is that recruitment thresholds are not unalterable, but instead can be increased or decreased in response to acute or chronic stimuli. For example, resistance training has been reported to increase MU

recruitment at lower recruitment thresholds (34). In comparison to slow rates of force development, high rates of force development result in MU recruitment at lower recruitment thresholds (35). Lastly, fatigue can acutely lower recruitment thresholds (12, 25). To date, the plasticity of MU recruitment thresholds have been largely neglected by investigations of resistance training on MU behavior during submaximal contractions. Thus, future studies should include analyses of $MUAP_{AMP}$ in relation to recruitment threshold and firing to better account for potential changes in MU recruitment patterns.

2.2 MU FIRING RATES

The relationship between firing rate and recruitment threshold

It is well accepted that increased excitation to the motoneuron pool will increase the firing rates of all MUs, however, the relationships between minimal, maximal and mean firing rates and recruitment threshold have been debated for over 60 years. Early investigations predominantly supported a positive linear relationship between firing rates and recruitment threshold. More recently, the majority of investigations have reported strong negative firing rate vs. recruitment threshold relationships. The conflicting findings are likely the result methodological differences.

Early support of the firing rate scheme in which low threshold MUs fire slower than high threshold MUs was provided by Eccles et al (36) and Kernell et al (37). Eccles et al (36) electrically stimulated the severed motoneurons of slow, red (crureus, caput mediale of triceps brachii and anconeus) and fast, white (vastus lateralis, rectus femoris, caput laterale and caput longum of triceps brachii) muscles to elicit action potentials. Analyses indicated that the smaller

motoneurons innverating the red muscle had significantly slower conduction velocities than the larger motoneurons innervating the white muscles. Additionally, the action potentials elicited by electrical stimulation in the red muscle motoneurons had longer after-hyperpolarization periods than did the action potentials elicited in the white muscle motoneurons. Of importance, neither recruitment threshold nor firing rate were measured in the investigation. Nonetheless, Eccles et al (36) hypothesized that the discharge rate of the motoneuron would be dictated by the duration of the after-hyperpolarization. Thus, smaller MUs with smaller motoneurons and longer after hyperpolarization periods would fire slower than larger MUs. Similarly employing electrical stimulation of severed cat motoneurons, Kernell et al (37) reported that the minimal and maximal MU firing rates were correlated with the duration of the after-hyperpolarization period.

Since these early investigations, several investigations in humans have reported lower firing rates in lower threshold MUs compared to higher threshold MUs (38-43). These findings were observed in the first dorsal interosseous (39, 42, 43), toe extensors (38), soleus (41) and tibialis anterior (40) in younger (40-43) and older (39, 42) subjects. Though these studies all reported significant positive relationships, the correlation were relatively low in many studies. For example, Barry et al (39) reported r^2 values of 0.28 and 0.26 for the relationships between peak and minimal firing rates and recruitment thresholds, respectively. Tracy et al reported an r^2 of 0.09 value for the relationship between the average firing rate and recruitment threshold. Most notably, Oya et al reported an r^2 value of 0.38 for the relationship between peak firing rate and recruitment threshold, but only after the highest threshold MUs had been disregarded. With the high threshold MUs included, the r^2 value was 0.001. In all of the investigations, EMG data was collected via intramuscular electrodes. Due to limited MU yield of intramuscular electrodes, only a very limited number of MUs were observed per contraction and thus all statistical analyses

were analyses were conducted on composite data. In several instances, data for a single subject was obtained from multiple contractions at different intensities.

Though Person and Kudina (44) first reported that low threshold MUs demonstrated the greatest firing rates, De Luca et al (45) was the first to demonstrate a significant negative correlation between peak firing rate and recruitment threshold. In this investigation, peak firing of deltoid and first dorsal interosseous MUs were recorded via intramuscular electrodes during triangular contractions featuring a linearly increasing slope immediately followed by a linearly decreasing slope. In both muscles at both intensities, composite relationships from multiple individuals demonstrated peak firing rates progressively decreased as recruitment threshold increased. An important distinction from the aforementioned investigations was that the composite relationships were built solely from contraction at the same relative intensity, with the 40% and 80% MVC data analyzed separately

De Luca and Hostage (46) followed up on previous investigations by analyzing the relationship between mean firing rate and recruitment threshold in the vastus lateralis, tibialis anterior and first dorsal interosseous at 20%, 50%, 80% and 100% MVC. The three muscle were included due to their differences in reported MU recruitment and average firing rate ranges. The vastus lateralis had previously been reported to demonstrate the greatest recruitment range and lowest average firing rates, whereas the first dorsal interosseous had been reported to demonstrate the small recruitment range and highest average firing rates. This investigation was the first to analyze MU firing rates and recruitment thresholds with the Delsys five-pin surface sensor array and surface EMG decomposition algorithm. The surface EMG decomposition yielded approximately 23 MUs per contraction, thus overcoming the major limiting of intermuscular electrodes, low MU yield. Equally innovative, the greater MU yield allowed for

the linear regression of MU firing rate against recruitment threshold for each individual contraction. In all three muscles at all four intensities, strong negative relationships were observed between the mean firing rate during the steady force region of an isometric trapezoidal muscle action and the recruitment threshold. The average r^2 value of correlations from individual MU relationships ranged from r^2 values 0.814 – 0.927, significantly higher than those values supporting the positive firing rate vs. recruitment relationship. When composite relationships were established for each muscle at each intensity, the r^2 values were still significantly higher, ranging from 0.603 – 0.843, with the majority between 0.733 and 0.810. As contraction intensity increased, MUs of all recruitment threshold increased their firing rates in agreement with common drive. De Luca and Hostage concluded that the negative relationship between firing rate and recruitment threshold is invariant and that increased excitation to the motoneuron pool will increase the firing rates of all MUs such that at any level of excitation lower threshold MUs will always fire faster than higher threshold MUs.

The negative relationship between firing rate and recruitment threshold, or the onion-skin firing scheme as it has been referred to (47), has been observed in numerous additional investigations (8, 12, 13, 25, 48-55). The findings are not muscle or age specific as a negative firing rate vs. recruitment threshold has been reported in the first dorsal interosseous (8, 25, 51, 52, 56), vastus lateralis (12, 48, 53-55), rectus femoris (54), abductor digiti minimi (50, 57), and abductor pollicis brevis (49) in young and aged subjects. Perhaps more importantly, the negative firing rate vs. recruitment threshold relationship has been observed with a variety of EMG techniques including Delsys surface sensor arrays (8, 12, 48, 51, 53, 54), high-density EMG sensor grids (49, 50, 55) and intramuscular electrodes (25, 52, 56, 57).

Though the predominance of research supports the negative relationship between firing rate and recruitment threshold, both firing rates schemes have physiological merit as discussed by De Luca and Contessa in simulation study comparing the onion skin firing to the "after-hyperpolarization firing scheme (58). If low threshold MUs were to fire slower than high thresholds, in accordance with their longer after-hyperpolarization periods, all MUs would achieve twitch fusion at maximal excitation. As a result, the after-hyperpolarization scheme would maximize force production. In contrast, in the onion skin scheme, high threshold MUs may never achieve twitch fusion, thus impairing maximal force production. However, the faster firing of low threshold MUs provides smoother force production throughout the majority of the force and input excitation range. Additionally, in the onion-skin scheme the most fatigue resistance MUs fire at the fastest rates and the most fatiguable MUs fire at the slowest rates. Thus the onion-skin scheme is likely to reduce fatigue.

The effects of resistance training interventions on maximal MU firing rates

The effect of resistance training on MU firing rates during maximal contractions is unclear. To date, four investigations have examined maximal MU firing rates before and after a short resistance training intervention, with three reporting an increase and the last reporting no change. In all of the studies, maximal firing rates were measured via intramuscular electrodes.

Unlike the other investigations, which examined maximal firing rates during isometric contractions, Van Cutsem et al (34) reported increased maximal firing rates during ballistic contractions of the tibialis anterior. The MU firing rates of five subjects (3 female 2 male) were tested before and after 12 weeks of training where subjects performed ten sets of ten ballistic

dorsiflexion contractions at 30-40% of MVC force. Due to the brief nature of the ballistic contractions, researchers compared the discharge frequencies using the first three interpulse intervals. Prior to training the discharge frequencies of first three interpulse intervals were 84.6 ± 1.9, 64.8 ± 2.0 and 59.2 ± 2.6 Hz whereas post training the discharge frequencies of first three interpulse intervals were 90.2 ± 2.1, 89.4 ± 2.5 and 89.2 ± 3.3 Hz. Therefore, the results of the investigation suggest that ballistic resistance training can increase maximal firing rates during brief, dynamic contractions

Patten et al (59) reported that resistance training increased maximal MU firing rates during isometric contractions of the abductor digiti minimi in both young and old subjects . MU firing rates were measured twice pre-training, with a significant increase maximal firing rates observed in both hands in both groups only 48 hours after the first testing visit. Researchers reported an 18.2% increase in the trained hand of the young subjects and a 29.5% increase in the trained hand of the older subjects. Following the second pre-training testing visit, all subjects performed six weeks of resistance training consisting of two sets of ten maximal voluntary isometric fifth finger abduction performed five times per week using their non-dominant hand only. Maximal firing rates were analyzed following two and 6 weeks of training. Interestingly, maximal firing rates declined in both groups from the second pre-training visit to the post-training visit in both the trained and untrained hands. Thus, the resistance training program did not elicit any increase in maximal firing rates as these increases occurred prior to the initiation of training.

Kamen and Knight (60) similarly measured the effects of resistance training on maximal firing rates of the vastus lateralis in young and old subjects. Like Patten et al (59), MU maximal firing rates were assessed twice pre-training, seven days apart and then after two weeks and six

weeks of resistance training. Training consisted of three sets of ten dynamic knee extensions at 85% of 1RM and three five-second maximal isometric contractions performed three times per week. A 19% increase in maximal firing rates was observed across both groups from the first to the second pre-training testing visit, however, no further significant increases in maximal firing rates were elicited by the training program. Researchers attributed the increased maximal firing rates to decreased antagonist activity, which could decrease reciprocal inhibition of the agonist muscle. Importantly, like the previous investigation by Patten et al (59), the reported increases in maximal firing rates were not induced by the training program.

Pucci et al (61) reported no change in maximal firing rates of the vastus lateralis in young men following a short isometric resistance training program. Maximal firing rates were assessed before and after 3 weeks of 3 training visits with three sets of 10 three second maximal voluntary contractions performed at each training visit. Unlike the studies by Patten et al (59) and Kamen and Knight (60), maximal firing rates were only assessed once before training.

Based on the investigations by Van Cutsem et al (34), Patten et al (62) and Kamen and Knight (60), increases in MU firing rates are widely reported as a common adaptation to resistance training. A more thorough analysis of these findings, however, yields significant skepticism as to the validity of these reports. The increases firing rates reported by Van Cutsem et al (34) were calculated using only the first four firings of the MUs, thus the findings may be more indicative of firing rates at recruitment, rather than firing rates during a sustained contractions. Secondly, the maximal firing rate increases reported by Kamen and Knight (60) and Patten et al (59) both occurred prior to the initiation of the resistance training interventions. Following the interventions, neither study observed a significant increase in maximal firing rates from the second pre-training measurement. As such, the early increase in maximal firing rates

reported in these two investigations may be due to subjects' unfamiliarity with maximal effort contractions. Importantly, neither study reported a familiarization session prior to the first measurement. Thus, the ability of resistance training to increase maximal MU firing rates is questionable.

The effects of resistance training interventions on MU firing rates during submaximal contractions

To date, six studies have investigated the effect of resistance training on MU behavior during submaximal contractions, with five reporting no change in firing rate behavior and one reporting an increase in firing rates following resistance training. Unlike maximal firing rates, firing rates during submaximal contractions have been investigated using both intramuscular and surface EMG decomposition

Three studies reported no influence of resistance training on firing rates during submaximal contractions using intramuscular electrodes. Rich and Cafarelli (63) analyzed MU firing rates in the vastus lateralis during submaximal contractions at 50% knee extension MVC. Firing rates were measured before and after an 8-week isometric resistance training program consisting of five sets of ten brief (3-5s) MVCs performed three times per week. No change in average MU firing rate measured during a 10s isometric contraction was observed following the training program. In addition to measuring maximal MU firing rates, the aforementioned Pucci et al (61) study also measured MU firing rates at 50 and 75% MVC in the vastus lateralis and observed no change in firing rates at either intensity following the three week resistance training program. Having reported no change in either maximal or submaximal firing rates, researchers

concluded that the primary neural adaptation resistance training was not an increase in MU firing rates. Despite reporting a significant increase in maximal firing rates in the vastus lateralis, Kamen & Knight (60) reported no change in firing rates at submaximal intensities of 10 and 50% MVC following the six week resistance training program.

In contrast with these investigations, Vila-Cha et al (64) reported that six weeks of resistance training increased MU firing rates of the vastus lateralis and vastus medialis obliquus during submaximal contractions at 30% MVC. In this investigation, the training protocol included three lower body exercises (the leg press, leg extension and leg curl) as well as four upper body exercises performed three times per week. The training volume and intensity were programmed according to a linear periodization model. Following the training program, researchers reported firing rate increases of 12.8 ± 4.7% in the vastus lateralis and 10.8 ± 4.9% in the vastus medialis obliquues during the 30% MVC contractions. Average firing rates during the 10% contractions were increased, but not significantly different from pre-training. Researchers concluded that their observation resistance-training induced firing rate increases may have been due to the duration of their intervention or the use of dynamic, instead of isometric training.

Training duration was likely not the cause of the opposing findings by Vila-Cha et al (64) as their training duration was six weeks long, similar to Kamen and Knight (60), longer than Pucci et al (3 weeks) (61) and shorter than Rich and Cafarelli (8 weeks) (63). Additionally, their findings were not likely due to the use of dynamic contractions as Kamen and Knight also employed a dynamic contraction based training protocol, while Pucci et al and Rich and Cafarelli employed isometric MVC training.

A more likely explanation for the contrasting findings of these investigations was the calculation of an average firing rate. In all four of these investigations, researchers calculated a single average firing rate of all observed MUs on an individual or group basis. Such a calculation ignores the recruitment thresholds of the observed MUs. As such, the observation of a greater number of low threshold MUs at a given time point, either by coincidence or due to changes in recruitment thresholds, could artificially increase the average MU firing rate.

To account for the influence of observed MU recruitment thresholds, both Beck et al (48) and Stock and Thompson (54) analyzed the influence of resistance training on MU firing rates in relation to recruitment threshold. Beck et al (48) analyzed MU firing rates in relation in the vastus lateralis before and after eight weeks of resistance training. In this investigation subjects performed trained three times per week. At each training session subjects performed bench press, leg press and leg extensions for three sets of ten to twelve repetitions at approximately 80% of their 1RM. Surface EMG was recorded via Delsys surface sensor arrays and decomposed to yield action potential trains for approximately 28 MUs per subject. Firing rates were regressed against recruitment thresholds and the slopes and y-intercepts of these relationships were statistically analyzed. Despite significant increases in lower body strength, no changes in the parameters of the firing rate vs. recruitment threshold relationships were observed. Beck et al concluded that the lack of change in the relationship parameters did not mean that the firing rate of an individual MU did not change, but instead that the simply that the relationship between firing rate and recruitment threshold was unchanged. As such, it is possible that a simultaneous increase in firing rate and recruitment threshold could have occurred.

Stock et al (54) employed similar methodology to study MU firing rates in relation to recruitment thresholds in the VL and RF. In addition to analyzing firing rates at the same relative

intensity, firing rates were also analyzed at the same absolute intensity as pre-training. Pre-training, relationships were analyzed at 50% MVC while at post-training, the relationships were analyzed at 50% of the pre-training and post-training MVCs. In this investigation, subjects trained twice per week for ten weeks. At each training session, subjects performed five sets of five repetitions with the maximal weight that could be performed using proper technique. Post-training, no differences in the firing rate vs. recruitment threshold slopes or y-intercepts were observed in either muscle at the same relative intensity. Surprisingly, no differences in the relationship parameters were observed at the same absolute intensity despite a significant strength increase.

With one exception, previous studies have demonstrated that short-term resistance training interventions do not affect MU firing rates during submaximal contractions. Given that resistance training can increase MU sizes via muscle fiber hypertrophy (65), the lack of changes in the firing rates suggest that the muscle fiber is more susceptible to adaptation than the motoneuron. As stated earlier, recruitment thresholds are modifiable and thus it is possible resistance training may simultaneously decrease firing rates while increasing recruitment thresholds thus preserving the relationship as discussed by Beck et al (48). Additionally, it is possible that the short durations of the training interventions were insufficient to elicit motoneuronal adaptations sufficient to alter the firing rate vs. recruitment threshold relationships. Thus, future investigations should conduct longer duration training studies or perform cross-sectional comparisons of highly resistance-trained and sedentary subjects.

Cross-sectional comparisons of MU firing rates in highly resistance-trained vs. non-resistance trained individuals

Given the possibility that the short duration of resistance training interventions may be insufficient elicit sufficient motoneuronal adaptations to alter the firing rate vs. recruitment threshold relationship, it is surprising that only two studies have compared the firing rates of highly resistance-trained individuals to those of less trained individuals in cross-sectional approaches. Though such designs would not be able to report a causal relationship between training and firing rate adaptations, they could at a minimum indicate whether longer-term training interventions are warranted.

De Luca et al (45) compared the deltoid and first dorsal interosseous MU firing rates of elite level powerlifters, long distance free-style swimmers, nationally known concert pianists and untrained, unskilled control subjects. Though the training history of the powerlifters was not reported, it is likely that they had an extensive training history given that were all national champions. Intramuscular electrodes were used to measure MU firing rate at recruitment and derecruitment during isometric, pyramidal contractions at 40% MVC. In comparison to swimmers, the highly resistance-trained powerlifts demonstrated significantly lower firing rates at recruitment but significantly higher firing rates at derecruitment in the deltoid. Additionally, the powerlifters, as well as the other two trained groups, demonstrated higher firing rates at derecruitment in the first dorsal interosseous than the control subjects. De Luca et al hypothesized that the MU firing rate differences between the swimmers and powerlifters was due to potentiation of fatigue resistant fibers. Possessing a greater percentage of fatigue resistant fibers in the deltoid, the swimmers would have likely experienced greater potentiation resulting in the sharper decline in firing rates from recruitment to derecruitment as observed in the

investigation. This phenomenon was also stated to explain the difference between the trained and control subjects. Thus, this early cross-sectional analysis provides some support for the hypothesis that firing rate adaptations are a long term adaptation to resistance training.

Vastus lateralis MU firing rates of highly resistance trained and highly endurance trained individuals were compared by Herda et al (66). Surface EMG signals were collected via a surface sensor array during isometric trapezoidal contractions at 40% and 70% MVC and decomposed to yield firing rates and recruitment thresholds for the observed MUs. The slopes and y-intercepts of the mean firing rate vs. recruitment thresholds relationships were analyzed. Resistance trained subjects demonstrated similar slopes but lower y-intercept values compared to the endurance trained subjects at each intensity. These findings suggest that resistance trained subjects demonstrated universally lower firing rates across the recruitment threshold spectrum than their endurance trained counterparts. Herda et al. postulated that the differences in firing rate behavior may be attributed to differences in myosin heavy chain composition resulting in greater potentiation in the resistance trained subjects. Of note, no controls subjects were included in the investigation, thus it is impossible to determine if the differences between the two groups represent adaptations to resistance training, endurance training or both forms of training.

2.3 MOTOR UNIT RECRUITMENT AND FIRING RATES DURING REPETITIVE CONTRACTIONS

Motor units are not independently controlled by the central nervous system, but instead by the excitation to motoneuron pool, or common drive (47, 67). Thus increased excitation to the motoneuron pool will recruit larger MUs in accordance with the size principle and

simultaneously increase the firing rates of active MUs. During repetitive contractions, the excitation to the motoneuron pool necessary to sustain a given force can be influenced by fatigue and twitch potentiation.

Fatigue is associated with the accumulation of inorganic phosphate and H+, reducing overall force production via a reduction in Ca+ sensitivity and the force produced per crossbridge cycle (68). As a result of the attenuated force produced by a given MU, excitation to the motoneuron pool is increased, to increase firing rates and MU recruitment (25, 53, 69, 70). Recent investigations using decomposition EMG have provided significant insight into the effects of fatigue at the individual MU level (12, 13).

Contessa et al measured $MUAP_{AMPS}$, firing rates and recruitment thresholds from vastus lateralis MUs during repeated 54s long isometric knee extension contractions at 30% MVC (12). Expression of the firing rates relative to the $MUAP_{AMPS}$ indicated that as the MUs fatigued, MUs of the same size fired faster. Additionally, analysis of $MUAP_{AMPS}$ relative to recruitment threshold revealed that MUs were recruited at progressively lower recruitment thresholds as fatigue increased. This reduction in recruitment threshold resulted in greater MU recruitment to produce the same force.

In a follow up study, Contessa et al similarly measured MU activity during repetitive fatiguing 50% MVC contractions of the first dorsal dorsal interosseous (13). Muscle activation in the flexor carpi radialis, extensor carpi radialis and pronator teres muscles was also measured as activation of these forearm muscle may contribute to force production during fatiguing index finger abductions. Similar to the previous study, Contessa et al observed an increase in first dorsal interosseous EMG amplitude was observed concurrent with an increase in firing rates in

agreement as fatigue increased. During the later portion of the fatiguing contractions, however, a decrease in first dorsal interosseous EMG amplitudes and MU firing rates was observed. Concurrent with the decreased activity in the first dorsal interosseous, increased muscle activation was observed in all three forearm muscles. These findings suggest individuals may alter their force production strategy during fatigue by reducing activation of the fatigued muscle and increasing activation of concurrently active muscles.

Together these studies indicate that fatigue is associated with increased excitation to the motoneuron pool, increasing MU firing rates and recruitment. Fatigue does not reduce the firing rates of the active MUs, as had been reported by some previous investigations (71, 72). These previous reports of fatigue induced reductions in MU firing rates were likely the result of the altered force production strategies or the calculation of composite average firing rates. The latter would artificially reduce firing rates due to greater recruitment of the higher-threshold, slower firing MUs.

Previous contractile activity can transiently phosphorylate the myosin regulatory light chains, temporarily increasing MU twitch forces in a phenomenon known as twitch potentiation (73-75). In contrast with fatigue, twitch potentiation can decrease the required excitation to the motoneuron pool required to produce a given force (69, 76-78).

Klein et al (77) demonstrated the influence of twitch potentiation on MU firing rates in the triceps brachii. Isometric ramp and hold contractions were performed at 10%, 20% and 30% MVC, before and after a 75% MVC 5s conditioning contraction. Following the conditioning contraction peak evoked twitch force increased approximately 42% and mean firing rates decreased. Researchers found that the change in twitch torque were significantly correlated with

the decreases in firing rate. Thus, potentiation can significantly reduce the excitation needed to produce a given force.

2.4 RESISTANCE TRAINING INDUCED VASTUS LATERALIS HYPERTROPHY

Muscle hypertrophy, an increase in the size of a muscle and its component muscle fibers, is a commonly observed adaptation to resistance training. Resistance training induced muscle hypertrophy has been analyzed via a multitude of methodologies including measurements of muscle cross-sectional area (CSA), muscle thickness and muscle volume using MRI, computed tomography, and ultrasound imaging. Using panoramic ultrasound imaging, the entire cross-section of the vastus lateralis can be captured with a single image. To date, four investigations have employed panoramic ultrasound imaging to quantify the changes in vastus lateralis cross-sectional area elicited by resistance training interventions in untrained men.

Walker et al observed a ~17% increase in vastus lateralis CSA after 10 weeks of whole body resistance training. Subjects trained twice per week with 12 machine exercises (3 which targeted the knee extensors) performed at each session, The training program followed a linear periodization model beginning with two to three sets of 12-14 repetitions at 60-70% 1RM and ending with three to four sets of 8-10 repetitions at 75-85% 1 RM. After completing the 10-week training program, subjects subsequently performed the same 10 week program a second time, however no further increases in vastus lateralis CSA were observed.

Boone et al observed a 16.3 ± 7.6% increase in the vastus lateralis CSA of subjects who did not receive a protein supplement in addition to the training program. The CSA was only slightly higher in the group that received the supplement 16.8 ± 8.8%.

Subjects trained three times per week for four weeks. Each training session included 3 sets of 8 maximal effort unilateral countermovement jumps, and three sets of ten repetitions at 80% 1RM in both the leg press and leg extension.

Following a 10 week resistance training program, Damas et al (79) observed a ~10.4% vastus lateralis CSA increase. Subjects performed three sets of leg press and three sets of leg extensions each for approximately 9-12 repetitions at each of the two weekly training sessions. Intensities were adjusted between sets so that concentric failure occurred within the desired repetition range.

Lastly, the aforementioned study by Pope et al (9) observed an average vastus lateralis CSA increase of 13.7%, concurrent with increases in high threshold MU sizes. Though not specific to the vastus lateralis, or measurement via ultrasound a recent review by Wernbom et al (80) found that of the average quadriceps CSA increase following 44 training interventions ranging from 14 days to 79 days in duration was approximately 8.5% (range: 1.1 – 17.3%).

2.5 THE EFFECTS OF RESISTANCE TRAINING ON VOLUNTARY ACTIVATION

Voluntary activation refers to the ability to recruit the motor units of a muscle via descending drive. Measured via the interpolated twitch technique, voluntary activation quantifies the proportion of maximal force that can be produced via voluntary contraction of the muscle (81). Two investigations have investigated whether resistance training can increase voluntary activation of the leg extensors in untrained subjects.

In addition to investigating whether resistance-training elicited strength gains were due to increases in maximal firing rates, Pucci et al (61) also sought to determine if strength gains could

be explained by greater motor unit recruitment. Prior to the training intervention both the training group and controls demonstrated high voluntary activation of the knee extensors as measured via the interpolated twitch technique (Training: 95.7 ± 1.83%; Control: 94.3 ± 0.997%). Following the three-week training intervention consisting of repeated knee extensor maximal voluntary isometric contractions, both groups demonstrated a small but significant increase in voluntary activation (Training: 98.4 ± 0.658%; Control: 96.8 ± 1.3%). Researchers concluded that the increase in voluntary activation indicates increased ability to recruit MUs as a result of resistance training, however, given the similarity of the increase in the training (2.7%) and control (2.5%) groups, the contribution of resistance training to these improvements is questionable.

Knight et al (82) also sought to determine if resistance training could increase voluntary activation, albeit in both young and older adults. Voluntary activation was measured twice before the resistance training protocol, after two weeks of training and again after six weeks of training. Similar to the Pucci et al (61) investigation, voluntary activation was already high (>95%) in both the young and older subjects. Both groups demonstrated approximately 2% increases in voluntary activation from the first baseline visit to the week six testing visit, with a 1.7% increase from the second baseline visit to week 6. Thus, the findings of Pucci et al (61) and Kamen et al (61) suggest that resistance training may elicit small improvements in voluntary activation, however the small improvements likely do not explain the larger increases in maximal force.

Voluntary activation has also been used to determine whether resistance training can decrease the neural cost to produce a given absolute force. Jenkins et al (83) measured voluntary activation at 10 - 80% of the pre-training MVC in 10% MVC increments before and after a six week resistance training protocol. Following training, the high intensity was able to produce each

of the 10% MVC increments with reduced voluntary indication, indicating the same absolute forces could be produced with reduced descending drive.

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3. DIFFERENCES IN THE MOTOR UNIT FIRING RATES AND AMPLITUDES IN RELATION TO RECRUITMENT THRESHOLDS DURING SUBMAXIMAL CONTRACTIONS OF THE FIRST DORSAL INTEROSSEOUS BETWEEN CHRONICALLY RESISTANCE TRAINED AND PHYSICALLY ACTIVE MEN

3.0 ABSTRACT

Previous investigations report no changes in motor unit (MU) firing rates during submaximal contractions following resistance training. These investigations did not account for MU recruitment or examine firing rates as a function of recruitment threshold (REC). Therefore, MU recruitment and firing rates in chronically resistance trained (RT) and physically active controls (CON) were examined. Surface electromyography signals were collected from the first dorsal interosseous (FDI) during isometric muscle actions at 40% and 70% maximal voluntary contraction (MVC). For each MU, force at REC, mean firing rate (MFR) during the steady force, and MU action potential amplitude ($MUAP_{AMP}$) were analyzed. For each individual and contraction, the MFRs were linearly regressed against REC, whereas, exponential models were applied to the MFR vs. $MUAP_{AMP}$ and $MUAP_{AMP}$ vs. REC relationships with the y-intercepts and slopes (linear) and *A* and *B* terms (exponential) calculated. For the 40% MVC, the RT had less negative slopes ($p=0.001$) and lower y-intercepts ($p=0.006$) of the MFR vs. REC relationships and lower *B* terms ($p=0.011$) of the $MUAP_{AMP}$ vs. REC relationships. There were no differences in either relationship between groups for the 70% MVC. During the 40% MVC, the RT had a smaller range of MFRs and $MUAP_{AMPS}$ in comparison to the CON, likely due to reduced MU recruitment. The RT had lower MFRs and recruitment during the 40% MVC that may indicate a leftward shift in the force-frequency relationship, and thus require less excitation to the motoneuron pool to match the same relative force.

3.1 INTRODUCTION

Previous studies have reported that short term resistance-training (1 to 12 weeks) can elicit neuromuscular adaptations at the motor unit (MU) level including changes in recruitment and firing rates. Although maximal MU firing rates have been consistently shown to increase with resistance training (1-4), the effect on firing rates during submaximal contractions is less clear. While firing rate increases (5) and decreases (2) have been observed, most short-term resistance training investigations have reported no change in firing rates during submaximal contractions (1, 3, 6, 7). These findings may be due to analysis of firing rates without accounting for potential changes in MU recruitment, or resistance-training interventions of insufficient length. Therefore, the examination of firing rates in conjunction with recruitment patterns in highly resistance trained individuals is warranted. Assessing MU recruitment may provide an explanation for the changes, or lack thereof, of firing rates as a result of resistance training. In addition, assessing firing rates during submaximal contractions can provide an assessment of the force-frequency relationship. Resistance training related-increases in MU twitch forces may cause a leftward shift in the force-frequency relationship and, thus, could reduce muscle activation to produce the same relative submaximal force.

In contrast to these short-term interventions, there is limited evidence indicating that long-term resistance training (> 4 years) (8, 9) can alter MU firing rates. De Luca et al. (8) reported a reduced firing rate decline from recruitment to derecruitment and greater firing rates at derecruitment in powerlifters in comparison to untrained individuals for the first dorsal interosseous (FDI) and deltoid muscles, however, the authors did not report firing rates during steady force nor account for recruitment thresholds of the observed MUs. Herda et al. (9) reported significant differences in firing rates in relation to recruitment thresholds between

chronically resistance trained (> 4 years) and aerobically trained (> 3 years) individuals, however, untrained controls were not included in the investigation. Thus, the effects of chronic resistance training on MU firing rates are not well understood. Specifically, the influence that changes in MU recruitment patterns may have on firing rates is unclear.

Previous investigations have not accounted for the impact of changes in MU recruitment on firing rates. By analyzing firing rates in relation to recruitment threshold, the influence of potential changes in MU recruitment are accounted for, and threshold specific changes in firing rates can be observed. The effect of MU recruitment-related changes on firing rates can be further elucidated by analyzing the motor unit action potential amplitudes ($MUAP_{AMPS}$), an indirect assessment of MU size (10-13). When expressed relative to recruitment threshold, $MUAP_{AMPS}$ can identify the size of active MUs, and determine if an intervention accelerates or delays the recruitment of larger MUs (13). Furthermore, analysis of firing rates in relation to $MUAP_{AMP}$ could identify size specific differences in MU firing rates. Thus, $MUAP_{AMP}$ analysis may provide additional insight into the effects of chronic resistance training on MU firing rates and recruitment. For example, the increase in observed $MUAP_{AMPS}$ from a lower to higher contraction intensity will provide insight on recruitment patterns which should coincide with changes in firing rates from a lower to higher contraction intensity.

Therefore, the purpose of this investigation was to determine if chronically resistance trained individuals possess altered MU firing rates as a function of recruitment threshold and $MUAP_{AMP}$ in comparison to physically active controls during submaximal contractions. The FDI was chosen as almost, if not the entire, motoneuron pool would be active during a 70% maximal voluntary contraction (14, 15). Furthermore, the FDI muscle of non-resistance trained physically active individuals would not undergo training-related adaptions as minimal stress would be

applied to the muscle unlike for resistance trained individuals. Muscle cross-sectional area (CSA) of the FDI was assessed to provide an indirect marker of hypertrophy as a result of chronic resistance training. Knowledge of the CSAs may improve the interpretation of the $MUAP_{AMP}$ results, as $MUAP_{AMPS}$ have been sensitive to hypertrophy as measured via CSA (11). We hypothesize that muscle fiber hypertrophy in the resistance-trained individuals will increase $MUAP_{AMPS}$ and twitch forces of the MUs in comparison to the non-trained individuals, which may result in a leftward shift of the force-frequency relationship for the resistance-trained individuals.

3.2 MATERIALS AND METHODS

Subjects

Eight chronically resistance trained men (RT; age: 23.0 ± 2.3 years; height: 178.4 ± 9.1 cm; weight: 100.3 ± 28.5 kg) and eleven physically active, but not chronically resistance trained men (CON; age: 22.0 ± 3.8 years; height: 181.4 ± 6.1 cm; weight: 77.1 ± 13.0 kg) completed this investigation. Prior to participating, all subjects completed a health history questionnaire. No subjects reported any neuromuscular conditions or musculoskeletal injuries that might impact the results of this investigation. Subjects were asked to abstain from coffee and other ergogenic aids on the day of testing. Informed consent was obtained from all individual participants included in the investigation. The chronically resistance trained subjects participated in structured high intensity resistance training programs for at least four years and trained between six and ten hours per week. Seven of the eight RT subjects reported squat one-repetition maximums of at least two times bodyweight. These individuals reported to consistently perform high intensity

multi-joint movements, such as, squats, deadlifts, presses and rows. In addition, according to previously research, it is expected that these individuals have undergone significant neuromuscular adaptions (8, 9, 16, 17). Of the eleven active controls, five reported three to fifteen hours of aerobic exercise per week, five reported three to nine hours of recreational sports participation, and five of the eleven active controls reported two to six hours per week of resistance training. However, none of controls reported consistent training for the previous two years. The University of Kansas institutional review board for human subjects research approved this investigation.

Isometric Testing

Isometric force testing of the FDI was performed with the subject seated at a desk, with the right hand pronated and resting on the desk's surface. The thumb restrained by a wooden stopper and the distal segment of the index finger was positioned atop and adjacent to an L-shaped metal bracket affixed to a force transducer (LC 202-100; Omegadyne, Sunbury, OH, USA), such that the thumb and index finger were positioned at a 90° angle. Velcro straps restrained the subject's right wrist, forearm and middle, ring and pinky finger. When instructed, the subjects abducted the right index finger against the force transducer.

Prior to the start of the experimental trial, subjects practiced the maximal voluntary contractions (MVC) and tracing of the isometric trapezoidal muscle actions with 3 minutes rest between muscle actions. To avoid fatigue, 30 minutes rest was given between practice attempts and the start of the experimental trial. The experimental trial began with three 3-second isometric MVCs of the FDI with 2-minute rest periods between each contraction (Figure 1). During each

MVC, strong verbal encouragement was provided. The peak force observed during the MVCs was used to determine the relative target force amplitude of the subsequent trapezoidal muscle actions. Each subject performed trapezoidal muscle actions at 40% and 70% MVC in a randomized order. Each trapezoidal muscle action consisted of a linearly increasing segment, a 12 second plateau at the target force and a linearly decreasing segment. Force increased and decreased at a rate of 10% MVC per second, thus the durations of the 40% and 70% MVC trapezoidal muscle actions were 20 and 26 seconds, respectively. An example of the trapezoidal muscle action is provided in Figure 2. During the trapezoidal muscle action, subjects were instructed to accurately trace the force template displayed on a computer monitor. Subject's force tracings were superimposed on the force template, providing real time feedback. Prior to data collection, subjects practiced the MVCs and trapezoidal muscle actions to ensure accurate force tracings.

During each isometric muscle action, force (N) signals were recorded via an NI cDAQ (National Instruments, Austin, TX USA). Signals were recorded with a sampling frequency of 2,000 Hz and were low-pass filtered with a 10-Hz cutoff (zero-phase fourth order Butterworth filter). The highest 0.25 sec average of force that occurred during the three MVCs was selected as the peak force used for further statistical analysis. This peak force value was also expressed relative to the cross sectional area to measure the specific tension of the muscle.

EMG Recording

During the trapezoidal muscle actions, surface EMG signals were collected via a five-pin surface sensor array (Delsys, Boston, MA, USA), in which the pins were arranged at the corners and in the middle of a 5mm x 5mm square. The sensor was positioned above the FDI muscle belly and affixed to the skin via hypoallergenic tape. A reference electrode was placed over the right elbow. Prior to sensor placement, adhesive tape was repeatedly applied to sites to remove dry skin cells and then the sites were sterilized with alcohol. The signals from the electrode's four pairs of the pins were differentially amplified and filtered at a band-pass of 20-450 Hz using a Bagnoli EMG amplifier (Delsy21s, Boston, MA, USA). The EMG signals were sampled at 20 kHz and stored for subsequent decomposition and analysis.

EMG Decomposition

The four channels of raw EMG data collected by the five-pin sensor array were decomposed into their constituent motor unit action potential trains using the previously detailed Precision III algorithm (18-20). Only MUs that demonstrated ≥ 90% accuracy in a reconstruct-and-test procedure were included in the subsequent analyses. Custom-written software programs (LabVIEW 2015, National Instruments, Austin, TX, USA) were used to derive four parameters for each observed MU: recruitment threshold (REC), mean firing rate (MFR), $MUAP_{AMP,}$ and the MU action potential duration ($MUAP_{DUR}$). REC was determined as the average force of a 0.01 second epoch that began at the first firing instance of the MU expressed relative to %MVC. MFR was calculated as the average MU firing rate during the steady force epoch of the trapezoidal muscle action. $MUAP_{AMP}$ and $MUAP_{DUR}$ values were calculated by averaging the

peak-to-peak amplitude and time between peak-to-peak amplitudes of each of the four unique action potential waveform templates for each MU (11) using a custom-written LabVIEW program.

Ultrasound

Transverse ultrasound images of the right FDI were captured using NextGen LOGIQ e ultrasound console (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK) with a multi-frequency linear array transducer (Model 12L-RS; 5-13 MHz; 38.4 mm field-of-view). The images were generated using brightness mode imaging in LOGICview software. Equipment settings (skeletal muscle preset; scan depth: 2cm; gain: 28dB; frequency: 12MHz) were constant across all subjects.

Each subject's position during the collection of the ultrasound images was standardized. All subjects were examined while seated with their right hands resting pronated, palm down with the thumb and index finger positioned at a 70º angle. Ultrasound images were used to identify the origin and insertion of the FDI and the mid-point between the origin and insertion was marked. A transverse cross-sectional image was taken at the mid point with the probe oriented perpendicular to the 2nd metacarpal. Generous amounts of ultrasound gel were applied for each scan to ensure minimal pressure on the skin.

CSA and subcutaneous fat thickness were calculated from the ultrasound images using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). To calculate CSA, the image was scaled from pixels to cm using the straight-line function. The periphery of the FDI was outlined using the polygon function, with care taken to exclude the surrounding fascia. CSA was calculated within the outlined region. Subcutaneous fat thickness, measured as the distance

between the bottom of the cutaneous layer and the top of the muscle fascia, was calculated using the straight line function.

Statistical Analysis

Potential between-group differences in maximal force, CSA, specific tension and subcutaneous fat thickness were examined with independent samples t-tests. Linear models were applied to the MFR vs. REC and $MUAP_{DUR}$ vs. RT relationships for each contraction and individual with the slopes and y-intercepts used for statistical comparisons. An exponential model was applied to the $MUAP_{AMP}$ vs. REC and MFR vs. $MUAP_{AMP}$ relationships. In the $MUAP_{AMP}$ vs. REC model, $MUAP_{AMP} = Ae^{B(REC)}$, where the *A* term is the theoretical $MUAP_{AMP}$ for a MU recruited at 0% MVC, *e* is the natural constant, and the *B* term represents the growth coefficient of $MUAP_{AMP}$ with increments in REC. In the MFR vs. $MUAP_{AMP}$ model, $MFR = Ae^{B(MUAPAMP)}$, where the *A* term is the MFR scale factor, *e* is the natural constant, and the *B* term represents the rate of decay of MFR with increments in $MUAP_{AMP}$. Six separate two-way mixed-factorial ANOVAs (group [RT vs. CON] x contraction intensity [40% MVC vs. 70% MVC]) were used to analyze the slopes and y-intercepts of the MFR vs. REC relationship and the *A* and *B* terms of the $MUAP_{AMP}$ vs. REC and MFR vs. $MUAP_{AMP}$ relationships. When appropriate, follow-up analyses were performed using independent samples and paired samples t-tests with Bonferroni corrections (21, 22). Due a lack of significant relationships between $MUAP_{DUR}$ and REC, statistical analysis were not performed on the slopes and y-intercepts. Instead, the average $MUAP_{DUR}$ for each subject, at each contraction intensity, was analyzed with a two-way mixed-factorial ANOVA (group [RT vs. CON] x contraction intensity [40% MVC vs. 70% MVC]). In

addition, a correlation was performed between the slopes from the MFR vs. REC relationships and the *B* terms from the $MUAP_{AMP}$ vs. REC relationships. All data possessed a normal distribution (skewness and kurtosis < 2) except for the *B* terms from the MFR vs. $MUAP_{AMP}$ relationships from the 70% MVC (kurtosis = 3.191). The *B* terms were analyzed with an ANOVA and is considered robust to non-normal distributions (22). Alpha was set at $p < 0.05$. All statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, NY).

3.3 RESULTS

Maximal Force, CSA, Specific Tension and Subcutaneous Fat Thickness

Independent samples t-tests indicated no significant between group difference in maximal force ($p = 0.205$; RT: 29.4 ± 6.2 N; CON: 25.7 ± 5.8 N), but a significant differences in CSA ($p = 0.035$) with the RT (2.77 ± 0.46 cm^2) group possessing larger CSAs than the CON (2.40 ± 0.26 cm^2) group. Although maximal force was not significantly different between groups, the mean force for the RT group was approximately 14.2% greater than the CON group, which aligned with the 15.6% difference in CSA. However, there was no significant difference (1.6%) between groups for specific tension ($p = 0.884$; RT: 10.73 ± 2.16 $N \cdot cm^2$; CON: 10.90 ± 2.81 $N \cdot cm^2$). In addition, there was no between group difference was observed for subcutaneous fat thickness ($p = 0.271$; RT: 0.194 ± 0.070 cm; CON: 0.166 ± 0.038 cm) and, thus, the filtering effects of fat did not influence interpretation of potential $MUAP_{AMP}$ differences.

MU Analysis

Decomposition of surface EMG signals yielded 772 MUs, with 320 MUs observed for the 40% MVC and 452 MUs for the 70% MVC. Total number of MUs observed per individual and the REC ranges are presented in Table 1. Despite performing two isometric muscular contractions (i.e., 40 and 70% MVC), the lowest-threshold MUs (CON: REC < 4% MVC, RT: REC < 10% MVC) were not consistently observed in the present study (Table 1). For each individual, the MFR vs. REC (40% MVC: $r = 0.92 \pm 0.04$; 70% MVC: $r = 0.92 \pm 0.05$), $MUAP_{AMP}$ vs. REC (40% MVC: $r = 0.87 \pm 0.06$; 70% MVC: $r = 0.85 \pm 0.10$), and MFR vs. $MUAP_{AMP}$ (40% MVC: $r = 0.85 \pm 0.06$; 70% MVC: $r = 0.80 \pm 0.09$) relationships were significant for the 40% ($p \leq 0.015$) and 70% MVC ($p \leq 0.007$). Examples of individual MFR vs. REC, $MUAP_{AMP}$ vs. REC and MFR vs. $MUAP_{AMP}$ relationships are presented in Figure 3.

The majority of individual $MUAP_{DUR}$ vs. REC relationships were not significant for the 40% ($r = -0.045 \pm 0.306$) and 70% MVC ($r = 0.189 \pm 0.218$). Only 2 of 19 ($p \leq 0.05$, $r = 0.026 \pm 0.663$) and 3 of 19 ($p \leq 0.026$, $r = 0.519 \pm 0.076$) individual relationships were significant for the 40% and 70% MVC, respectively. The depth of the observed MU can be a confounding factor for analysis of $MUAP_{AMP}$ as greater depth can arbitrarily decrease the size of the $MUAP_{AMP}$ measured via surface EMG. Given that $MUAP_{DUR}$ is a factor of MU depth (23), the lack of significant correlations suggests that observed MUs were recorded at similar depths. Therefore, the positive relationship observed between $MUAP_{AMP}$ and REC were a due to an increase in the peak-to-peak amplitudes of the MUs, and not a difference in depth (10).

MFR vs. REC Relationships

For the slopes, there was a significant group x intensity interaction ($F(1,17) = 13.784$; $p = 0.002$). The independent samples t-tests indicated differences between groups for the 40% MVC ($t = 4.033$; $df = 17$; $p = 0.001$) but not for the 70% MVC ($t = 0.660$; $df = 17$; $p = 0.518$). For the 40% MVC, the RT group possessed less negative slopes than the CON group (Figure 4). Paired samples t-tests indicated no differences in the slopes between the contraction intensities for the RT ($t = 0.355$; $df = 7$; $p = 0.733$) group, however, the CON ($t = -4.576$; $df = 10$; $p = 0.001$) group had more negative slopes at the 40% than 70% MVC.

For the y-intercepts, there was a significant group x intensity interaction ($F(1,17) = 13.343$; $p = 0.002$). The independent samples t-tests indicated differences between groups for the 40% MVC ($t = -3.178$; $df = 17$; $p = 0.006$) but not the 70% MVC ($t = 0.387$; $df = 17$; $p = 0.704$). At 40% MVC, the RT group had lower y-intercepts than the CON group. Additionally, the RT group demonstrated lower y-intercepts at 40% than 70% MVC ($t = -4.886$; $df = 7$; $p = 0.002$). There were, however, no differences in the y-intercepts between contraction intensities for the CON group ($t = 0.461$; $df = 10$; $p = 0.655$).

For the 40% MVC, the slopes and y-intercepts suggest that the RT group had reduced MFRs for the lowest-threshold MUs and smaller decreases in the MFRs with increments in REC. The predicted MFR values demonstrate lower firing rates for the lowest-threshold MUs for the RT than the CON group, however, the higher-threshold MUs had greater observed (REC = 25-30% MVC) predicted (REC >30% MVC) firing rates (Figure 4). In comparison to the 40% MVC, the RT group had similar slopes but greater y-intercepts for the 70% MVC. In contrast, the CON group had no change in the y-intercepts but the slopes became less negative. Therefore,

both groups had similar slopes and y-intercepts at the 70% MVC and, thus, demonstrated similar firing rates at 70% MVC.

$MUAP_{AMP}$ vs. REC Relationships

For the *A* terms, there was a significant group x intensity interaction ($F(1,17) = 8.144$; $p = 0.011$). The independent samples t-tests indicated no differences between groups for the 40% MVC ($t = 0.818$; $df = 17$; $p = 0.425$) or the 70% MVC ($t = -1.858$; $df = 17$; $p = 0.081$). Paired samples t-tests indicated no differences in the *A* terms between the contraction intensities for the RT ($t = -0.177$; $df = 7$; $p = 0.864$) group, however, the CON group had lower *A* terms from the 40% compared to the 70% MVC ($t = -4.382$; $df = 10$; $p = 0.001$).

For the *B* terms, there was a significant group x intensity interaction ($F(1,17) = 13.250$; $p = 0.002$). The independent samples t-tests indicated between-group differences for the 40% MVC ($t = -2.844$; $df = 17$; $p = 0.011$) but not for the 70% MVC ($t = 0.212$; $df = 17$; $p = 0.834$). The RT group possessed lower *B* terms than the CON group (Figure 5). Paired samples t-tests indicated no differences in the *B* terms between contraction intensities for the RT ($t = 2.148$; $df = 7$; $p = 0.069$) group, however, the CON ($t = 8.387$; $df = 10$; $p < 0.001$) group had greater *B* terms for the 40% than 70% MVC.

For the CON group, the difference in *A* terms between contraction intensities was likely a function of differences in average lowest observed REC (40%: 7.70 ± 5.13%; 70%: 19.78 ± 8.21% MVC) rather than physiological phenomenon. At 40%, the *A* terms indicate no between-group difference in the size of the lowest threshold MUs, however, the *B* terms indicate reduced $MUAP_{AMP}$ growth with increases in REC for the RT group (Figure 5). The predicted $MUAP_{AMPs}$

demonstrate that as REC increased, so did the disparity in $MUAP_{AMP}$ between groups for a given REC. Similar to the MFR vs. REC relationships, there were no differences between groups for the 70% MVC.

MFR vs $MUAP_{AMP}$ Relationships

For the *A* terms, there was no significant group x intensity interaction ($F(1,17) = 1.918$; $p = 0.184$) nor significant main effect for intensity ($F(1,17) = 1.145$; $p = 0.300$) or group ($F(1,17) = 4.074$; $p = 0.060$). For the *B* terms, there was no significant group x intensity interaction ($F(1,17) = 0.358$; $p = 0.558$) nor main effect for group ($F(1,17) = 0.002$; $p = 0.962$). There was a significant main effect for intensity ($F(1,17) = 14.751$; $p = 0.001$) indicating a greater decrease in firing rates as $MUAP_{AMP}$ increased at 40% compared to 70% MVC (Figure 6).

$MUAP_{DUR}$ vs. REC Relationships

Since there were few significant $MUAP_{DUR}$ vs. REC relationships, the average $MUAP_{DUR}$ was calculated and analyzed with a two-way mixed-factorial ANOVA (group [RT vs. CON] x contraction intensity [40% MVC vs. 70% MVC]). There was no significant group x intensity interaction ($F(1,17) = 0.015$; $p = 0.905$) or main effect for group ($F(1,17) = 0.765$; $p = 0.394$). There was a significant main effect for contraction intensity ($F(1,17) = 18.890$; $p < 0.001$) with greater average $MUAP_{DUR}$ observed at 70% MVC (3.087 ms) than 40% MVC (2.638 ms). The lack of a significant group main effect indicates that MUs of similar depth were observed in each group and, thus, direct group comparisons are appropriate. The significant main effect for contraction intensity suggests that deeper MUs may have been observed during the

70% in comparison to the 40% MVC regardless of group. Subsequently, $MUAP_{AMPS}$ during the 70% MVC may be arbitrarily smaller as result of being recorded from deeper locations in the muscle. Of importance, the inclusion of the 40% and 70% MVCs in the same ANOVA model for the *A* and *B* terms from the $MUAP_{AMP}$ vs. REC and the MFR vs. $MUAP_{AMP}$ relationships should be interpreted with care as the $MUAP_{AMPS}$ might be different as a function of depth rather than physiological phenomenon.

Correlation Analysis

There was a significant correlation ($r = 0.81$, $p < 0.001$) between the slopes from the MFR vs. REC relationship and the *B* terms from the $MUAP_{AMPS}$ vs. REC relationship (Figure 7).

3.4 DISCUSSION

In the present investigation, differences in MU behavior were observed between chronically resistance-trained individuals and physically active, non-resistance trained individuals at 40% but not 70% MVC. Specifically, the RT group demonstrated a reduced MFR decline and $MUAP_{AMP}$ growth as REC increased. Furthermore, the change in firing rates from the lower- to higher-targeted forces was also different between groups. The RT group's reduced $MUAP_{AMP}$ growth during the 40% MVC, despite greater muscle CSAs in comparison to the CON group, likely indicates delayed MU recruitment.

The mean slope and y-intercept values of the MFR vs REC relationships at 40% MVC suggest MU behavior alterations in the RT group. The CON group demonstrated slopes and y-intercept values in accordance with those previously reported by Hu et al. (10), however, the RT

group demonstrated lower y-intercepts and less negative slopes. The RT group's reduced y-intercepts indicated that the lowest-threshold MUs for the RT group were firing approximately 35% slower than the CON group (RT = 22.61 pps; CON = 33.14 pps) at steady force. Although both groups demonstrated negative slopes from the MFR vs. REC relationships in accordance with the *Onion Skin Control Scheme* (10, 14, 24, 25), the RT group had a much slower rate of decline in MFRs with increments in REC. As a result of this slower decline in MFRs, the RT group demonstrate a smaller difference in MFRs between MUs recruited at 10% and 30% MVC (6.45 ± 3.95 pps) than the CON group (11.09 ± 3.34 pps). The smaller differences in MFRs between these RECs suggests a greater similarity (homogeneity) amongst the active MUs of the RT group (10). This increased homogeneity of active MUs may be the result of the RT group requiring fewer MUs to match the 40% MVC.

The observed $MUAP_{AMPS}$ in relation to REC further suggests greater homogeneity of active MUs in the RT group during the 40% MVCs. $MUAP_{AMP}$ has been correlated with the diameter of muscle fibers that comprise a MU (26), and thus provides an indirect measure of MU size. In addition, the slope of the $MUAP_{AMP}$ vs. REC relationships is an indicator of muscle hypertrophy (11) and atrophy (12). The lack of differences in the *A* terms suggested that the lowest-threshold MUs possessed similar $MUAP_{AMPS}$, however, the RT group had significantly smaller growth in $MUAP_{AMPS}$ with increments in REC. Due to this reduced growth, the RT group demonstrated a smaller difference in $MUAP_{AMP}$ between MUs recruited at 10% and 30% MVC than the CON group (RT: 0.50 ± 0.38 mV; CON: 0.80 ± 0.45 mV). As the RT group possessed larger FDI CSAs and demonstrated similar $MUAP_{AMP}$ vs. REC relationship parameters during the 70% MVC, it is unlikely that the RT group possessed smaller high

threshold MUs. Instead, the RT group's reduced $MUAP_{AMP}$ growth during the 40% MVC likely indicates a delayed recruitment of the larger MUs.

Evidence that a similar mechanism resulted in the attenuated decrease in MFRs and increases in $MUAP_{AMPS}$ in relation to REC at 40% MVC is provided by a strong correlation (between the slopes of the MFR vs. REC relationships and *B* terms of the $MUAP_{AMP}$ vs. REC relationships (Figure 7). The correlation indicated that at 40% MVC, individuals with the greatest decline in MFRs also had the greatest increase in $MUAP_{AMP}$ in relation to REC. The smaller range of MFRs and $MUAP_{AMPS}$ during the 40% MVC was likely due to a smaller active MU pool in the RT group, potentially due to lower descending drive resulting in reduced excitation to the motoneuron pool. The level of relative excitation is the primary determinant of both the number of recruited MUs and the firing rates of the active MUs. Therefore the lower firing rates of the lowest-threshold MUs suggest reduced excitation to the motoneuron pool for the RT relative to the CON group. Lower excitation to the motoneuron pool for the RT could be due to hypertrophy of muscle fibers. Such hypertrophy could increase the twitch forces of the lowest-threshold MUs. An increased fiber area and greater twitch forces of lower-threshold MUs would shift the force-frequency relationship to the left, thus requiring reduced muscle activation (reduced MU firing rates and recruitment) to produce the same relative force. A similar mechanism has been suggested to explain age-, fatigue-, and potentiation-related differences in MU recruitment (13, 27, 28). The reduced MU recruitment results in more homogenous MFRs and $MUAP_{AMPS}$ of the active MUs, as observed in this investigation. Thus, a novel finding of the present study is that chronic resistance training-related changes in firing rate behavior during submaximal contractions is likely due to lower excitation resulting in reduced MU recruitment.

Reduced descending drive, or lower excitation, during the 40% MVC would explain the increase in the MFRs of the lowest-threshold MUs to achieve the 70% MVC for the RT group. Firing rates do not increase linearly with increasing excitation, but instead demonstrate a rapid initial increase followed by a plateau with minimal further firing rate increases (8, 25, 29-31). As a result of lower excitation, the lowest-threshold MUs of the RT group may not have reached the firing rate plateau. Therefore, the additional excitation to achieve the 70% MVC significantly increased the MFRs of the lowest-threshold MUs as evidenced by the significant increase in y-intercepts from 40% to 70% MVC for the RT group. The increase in y-intercepts from 40% to 70% MVC, without a change in slope, suggests firing rates increased for all MUs active during the 40% MVC. In contrast, the CON group likely attained the steady firing rate plateau at 40% MVC, thus further increases in excitation elicited minimal increases in the MFRs of the lowest-threshold MUs as indicated by the lack of change in y-intercepts. The change in MFR vs. REC slopes from 40% to 70% MVC suggests that the greater excitation did increase firing rates of the larger higher-threshold MUs in the CON group.

In contrast to the 40% MVC, there were no differences in the MFR and $MUAP_{AMP}$ vs. REC relationships between the RT and CON groups during the 70% MVC. Although differences in excitation may have persisted at 70% MVC, these differences would only minimally impact MU recruitment given that maximal MU recruitment generally occurs just prior to 70% MVC (14, 15). In addition, at 70% MVC the lowest-threshold MUs of both groups likely reached the firing rate plateau, therefore, small differences in excitation would have minimal effect on MFRs of these MUs. Of note, but not directly statistically tested, Figures 3 and 5 suggest a rightward-shift in the $MUAP_{AMP}$ vs. REC relationships during the 70% MVC for the RT group in comparison to the CON group. The rightward-shift of the $MUAP_{AMP}$ vs REC relationship is

indicative of an extended recruitment range may provide further evidence that the RT group achieved similar relative forces with reduced MU recruitment.

Previous investigations have reported conflicting findings regarding the effects of resistance training on MU firing rates during submaximal contractions, including increases (5), decreases (2), and no change in firing rates (1, 3, 6, 7). The previous methods used to analyze MU firing rates may have led to these divergent findings. For the 40% MVCs, the RT group had reduced firing rates of the lower-threshold MUs but greater firing rates of the higher-threshold MUs. As a result of these non-uniform differences in firing rates, examining averaged MFRs across MUs without regard to REC would mask these differences between the RT (13.8 ± 2.4 pps) and CON (16.1 ± 2.7 pps) groups. Averaging firing rates across MUs without accounting for REC during submaximal contractions is a likely explanation for the lack of resistance training-related differences reported previously (1, 3, 6). Thus, the current investigation supports previous recommendations against the calculation of a single average firing rate value (13). In addition, accounting for recruitment patterns is necessary for thorough interpretation of potential firing rate changes during submaximal contractions.

In summary, a novel finding of the present study was that differing MU recruitment patterns contributed to the altered firing rate behavior during the 40% MVC for the RT in comparison to the CON group. Furthermore, the ability to match the same relative force with fewer activated MUs and lower firing rates would reduce the required voluntary effort and serve as a fatigue protecting mechanism during a moderate intensity submaximal contractions.

3.5 TABLES

Table 1. Total number of motor units (MU), mean (± SD) MUs per subject, observed mean lower (Low)- and higher (high)-threshold recruitment threshold (REC, expressed as a percentage of maximal voluntary contraction [%MVC]), and REC range from the 40% and 70% MVC for the chronically resistance trained (RT) and physical active non-resistance trained (CON) individuals.

		Total MUs	MUs per Subject	Low REC (%MVC)	High REC (%MVC)	REC Range (%MVC)
40% MVC	RT	133	17 ±4	4.3 ± 1.9%	35.2 ± 4.8%	31.0 ± 4.3%
	CON	187	17 ±5	10.2 ± 5.4%	28.1 ± 5.9%	17.9 ± 6.7%
70% MVC	RT	194	24 ± 5	23.5 ± 9.3%	62.4 ± 8.8%	38.8 ± 10.2%
	CON	258	23 ± 6	17.1 ± 6.4%	53.2 ± 6.6%	36.2 ± 9.4%

3.6 FIGURES

Figure 1. A representative three-second maximal voluntary contraction for a resistance-trained (RT) and control subject (CON).

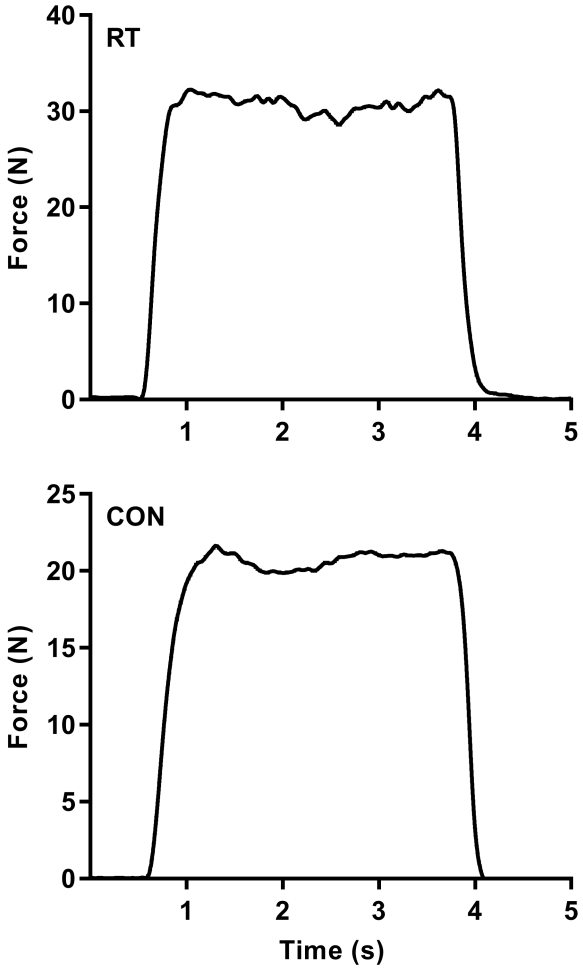


Figure 2. (Top) Motor unit (MU) firing rates during the course of an isometric trapezoidal muscle action. The lower-threshold MU (MU 3) maintains a higher firing rate than the higher-threshold MU (MU 10) throughout the contraction. (Middle) The four unique action potential waveforms of the selected MUs. The lower-threshold MU possess smaller peak-to-peak action potential amplitudes than the higher-threshold MU in all four channels. (Bottom) The mean firing rate vs recruitment threshold, MU action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold and mean firing rates vs. motor unit action potential size relationships for the subject.

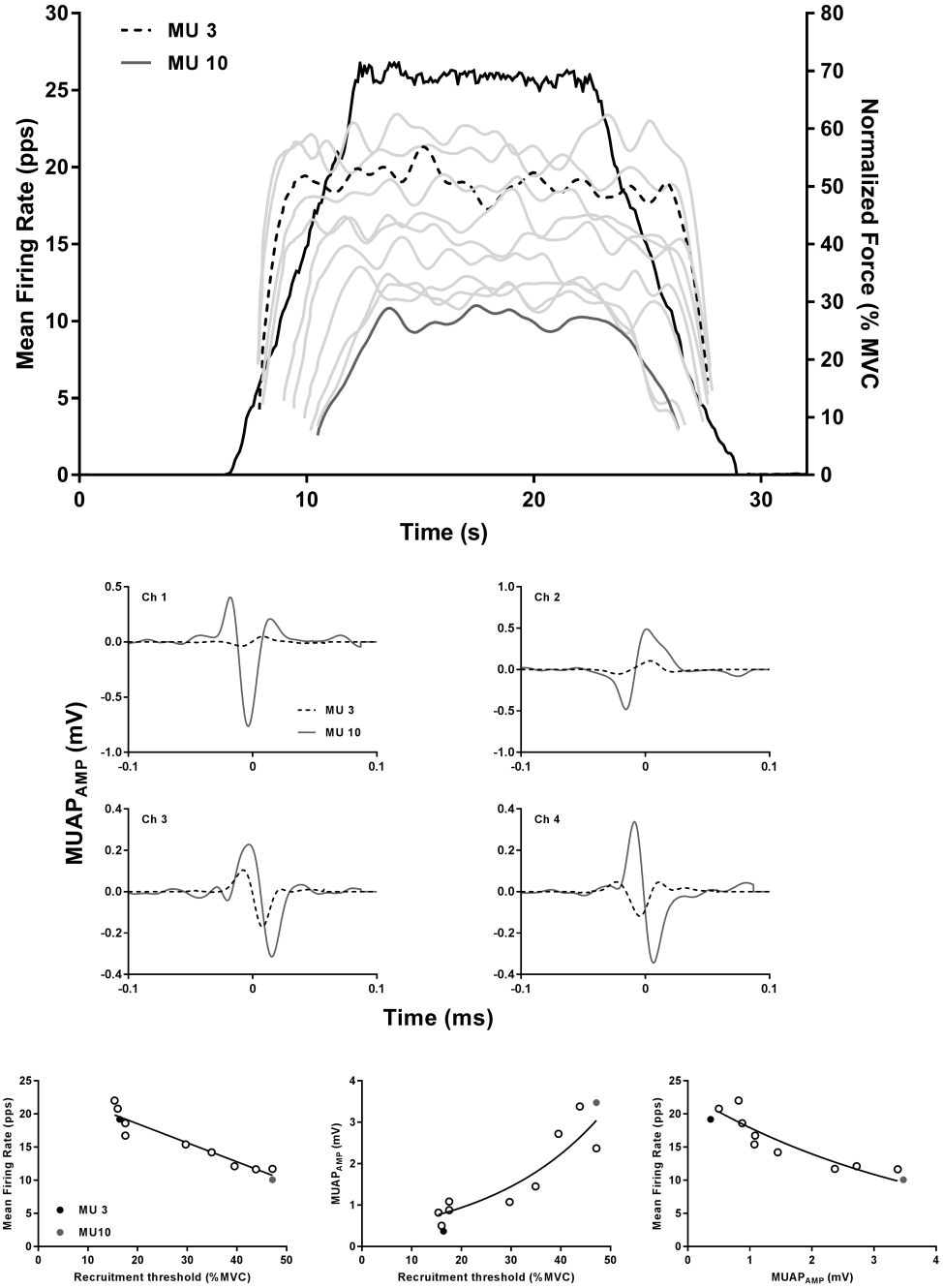


Figure 3. Representative mean firing rate vs. recruitment threshold (top), motor unit action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold (middle), and mean firing rate vs. $MUAP_{AMP}$ (bottom) relationships at 40% and 70% maximal voluntary contraction (MVC) for a resistance trained (RT) and control (CON) subject. Each data point represents an observed motor unit.

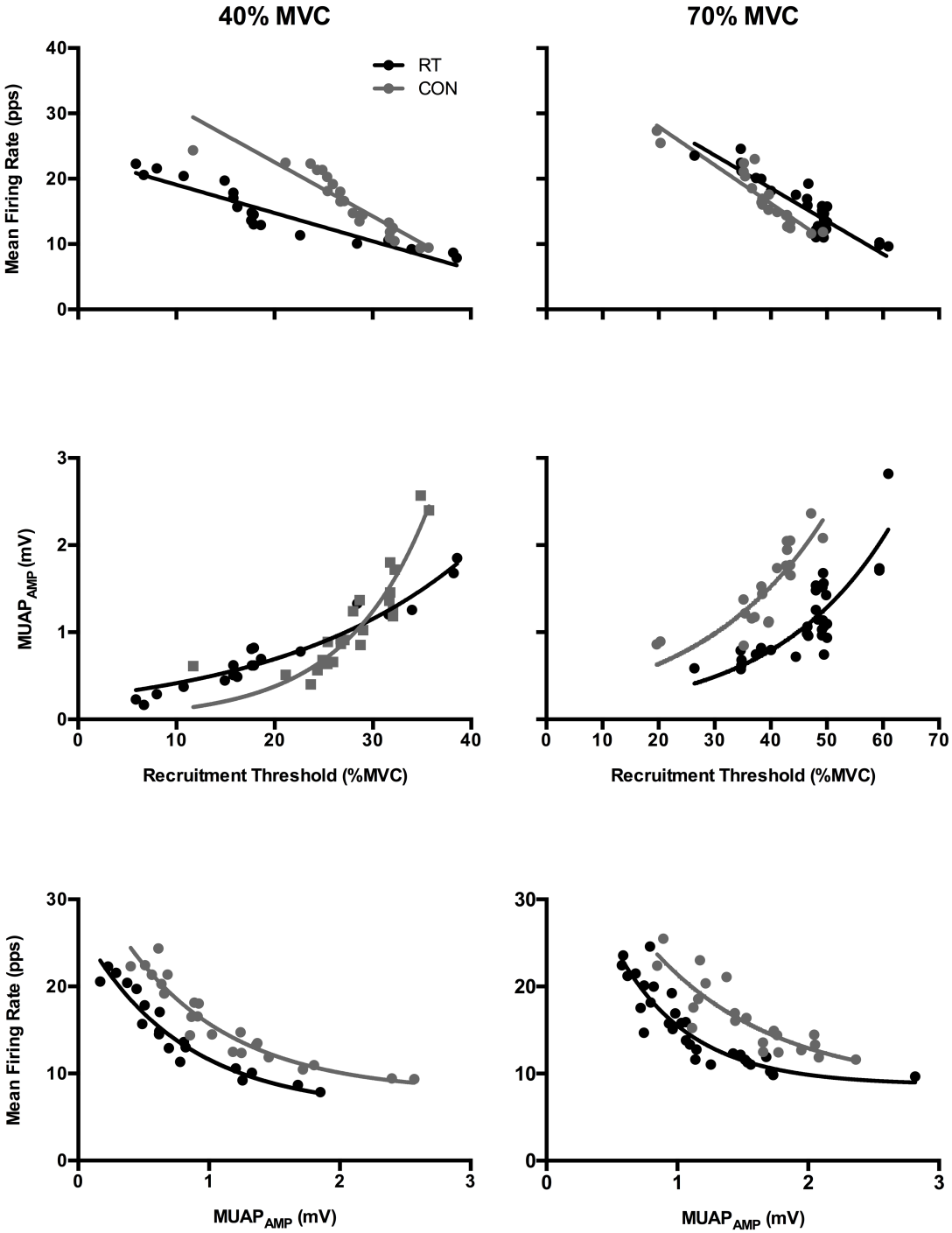


Figure 4. The individual y-intercept values (top), slope values (middle) and composite patterns (bottom) of the mean firing rate vs. recruitment threshold relationships. Horizontal bars represent the means and standard deviations for the respective groups.* indicates the resistance trained (RT) group was significantly different than the control (CON) group (slope: p=0.001; y-intercept: p=0.006). † indicates that the 70% maximal voluntary contraction (MVC) value is significantly different from the 40% MVC for that group (slope: p=0.001; y-intercept: p=0.002).

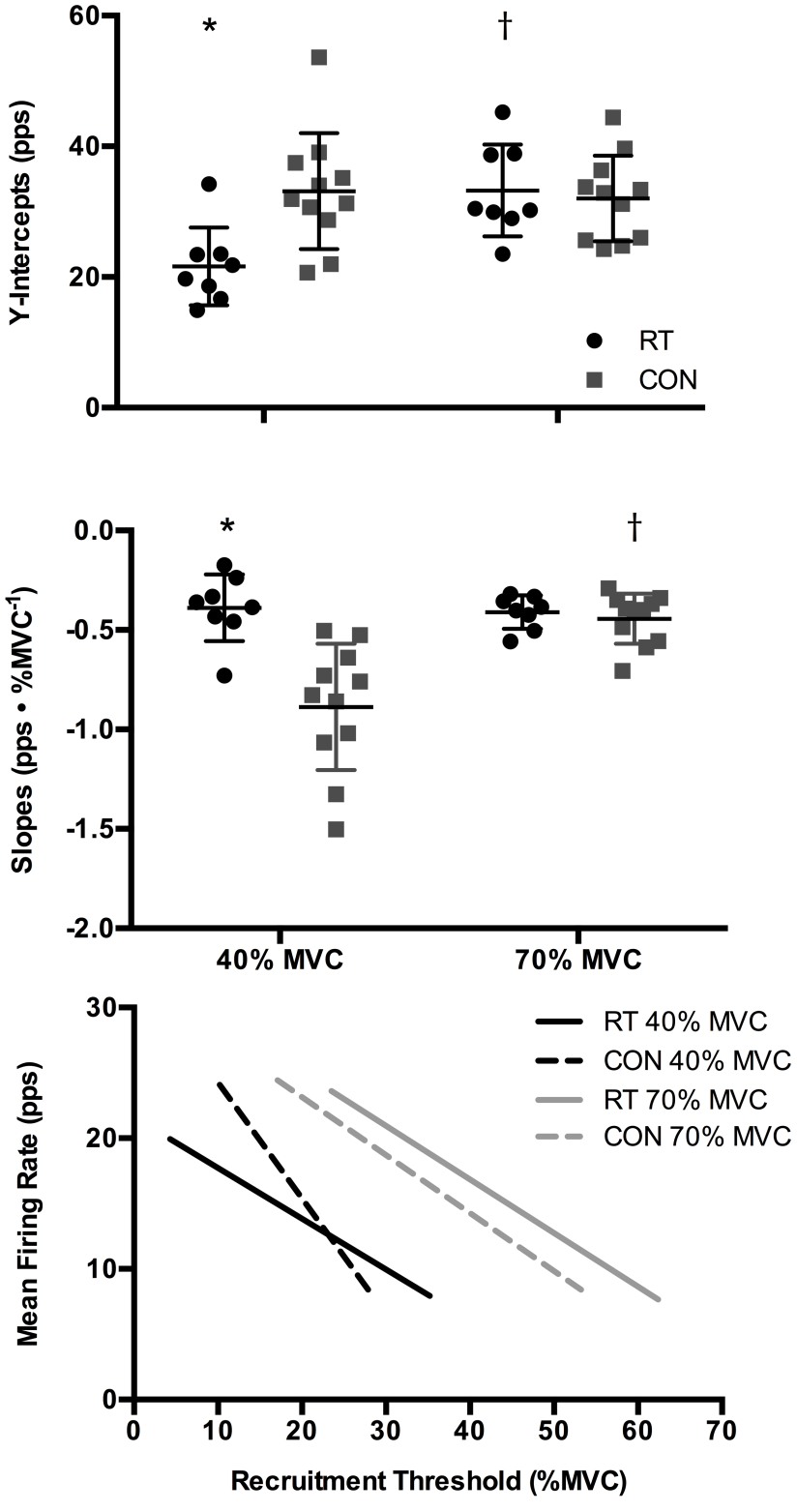


Figure 5. The individual *A* term values (top), *B* term values (middle) and composite patterns (bottom) of the motor unit action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold relationships. Horizontal bars represent the means and standard deviations for the respective groups.* indicates the resistance trained (RT) group was significantly different than the control (CON) group (*B* term: p=0.011). † indicates that 70% MVC is significantly different from the 40% MVC for that group (*A* term: p=0.001; *B* term: p<0.001).

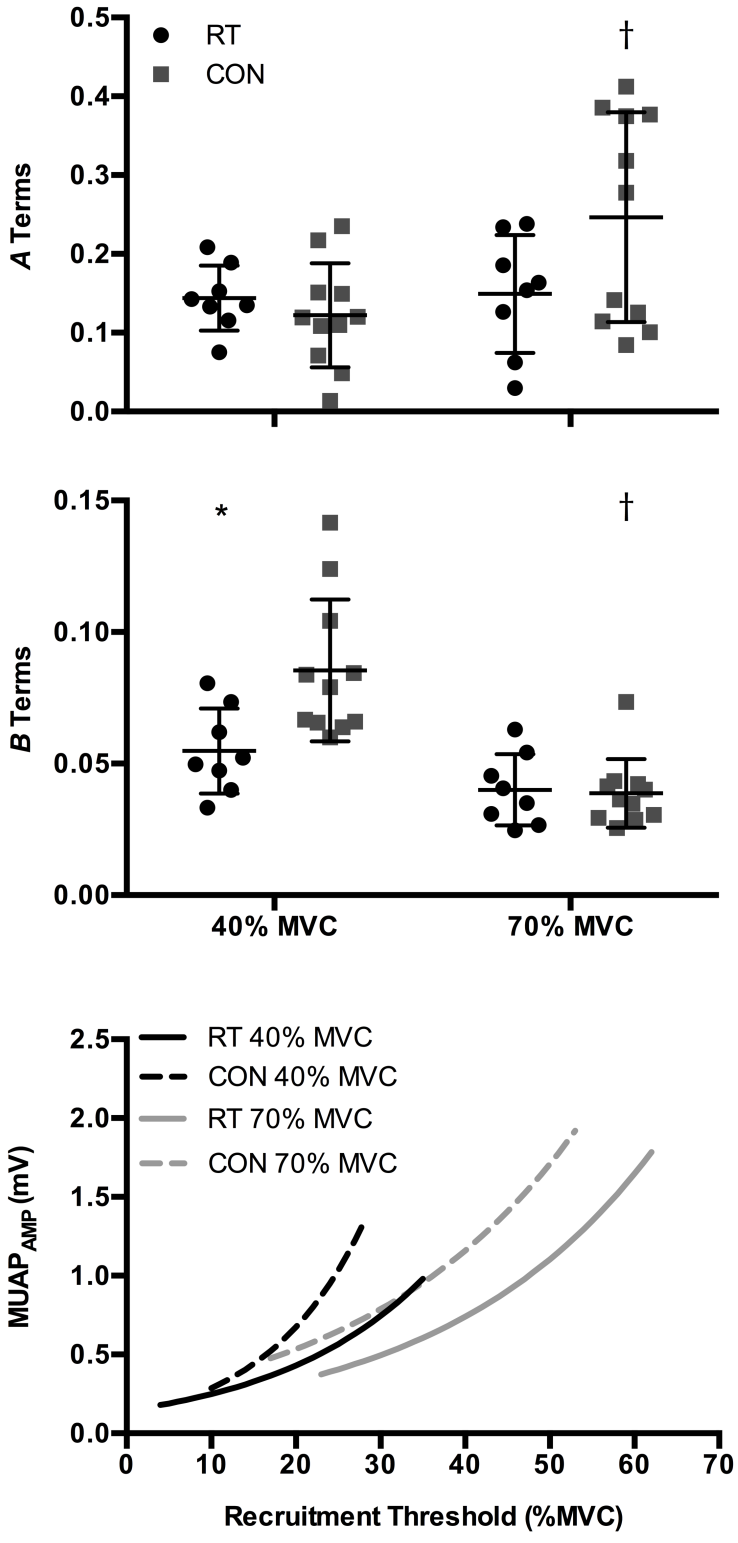


Figure 6. The individual *A* term values (top), *B* term values (middle) and composite patterns (bottom) of the mean firing rate vs. motor unit action potential amplitude ($MUAP_{AMP}$) relationships. Horizontal bars represent the means and standard deviations for the respective groups.

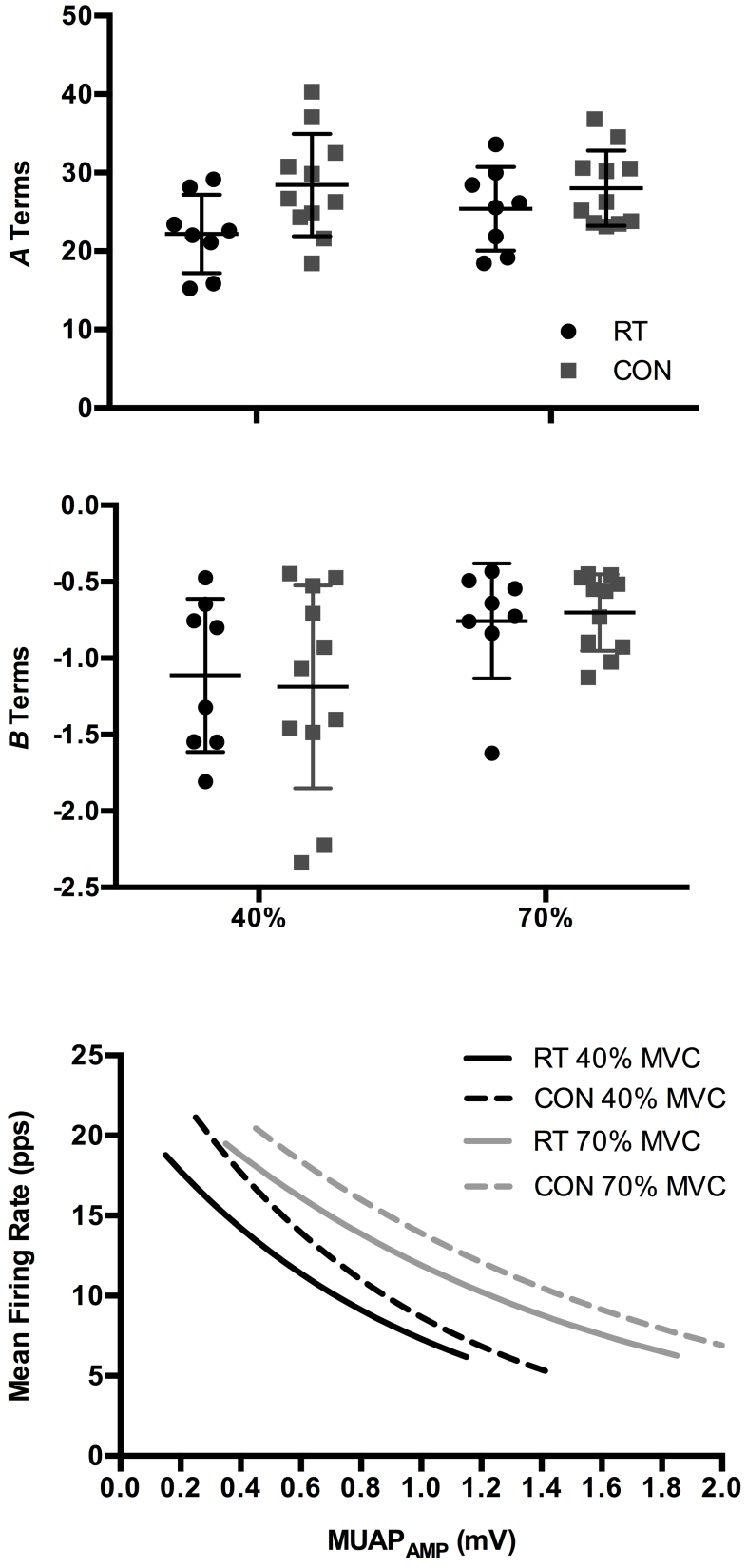
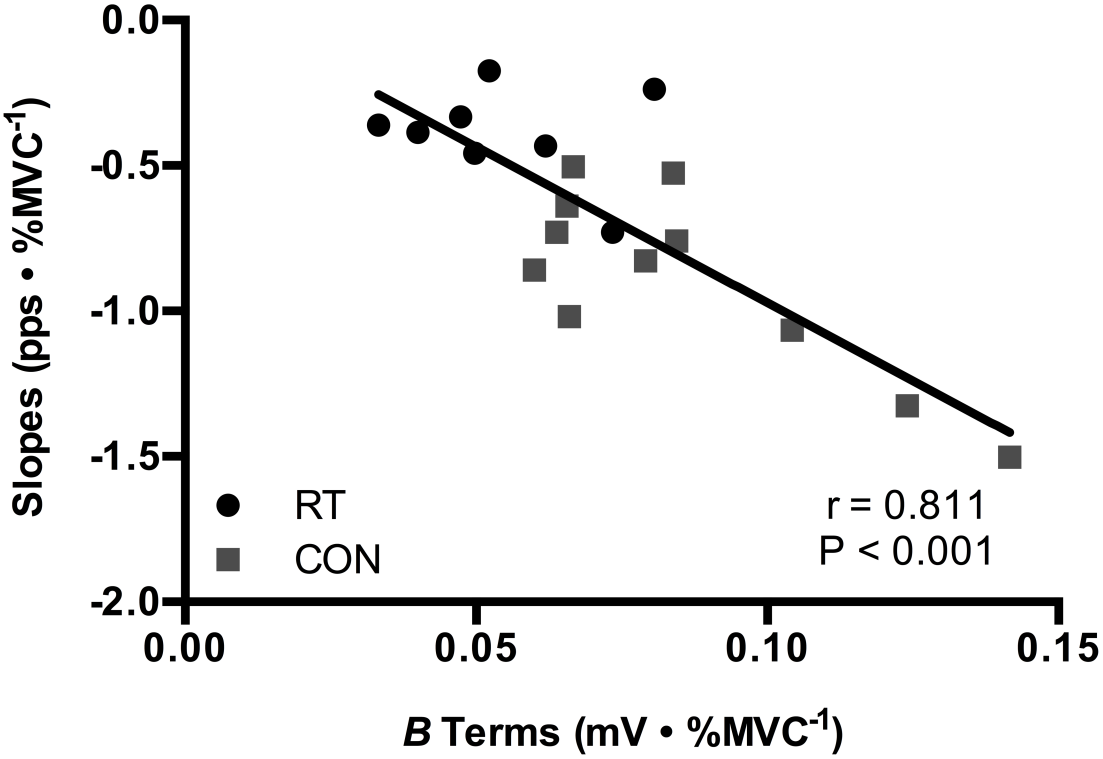


Figure 7. The correlation between slopes of the mean firing rate vs. recruitment threshold relationships and the *B* terms of the motor unit action potential size vs. recruitment threshold relationships. RT = Resistance trained; CON = Physically active control subjects.



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4. RESISTANCE TRAINING INCREASES MOTOR UNIT SIZES, BUT DOES NOT ALTER FIRING RATES

4.0 ABSTRACT

To examine the effects of an eight-week resistance-training program on the relationships between motor unit (MU) action potential amplitudes (AP_{AMPS}), mean firing rates (MFR) at steady torque, and recruitment thresholds (RT) of the vastus lateralis (VL) at 70% of maximal voluntary contraction (MVC). Sixteen males (age = 20.7 ± 1.9 years) completed an eight-week resistance-training program, while ten males (age = 19.1 ± 2.3) served as controls. Pre- and post-treatment, $MUAP_{AMP}$, MFR, and RT for each MU were analyzed from 70% MVCs. Linear regression models were fitted to the $MUAP_{AMP}$ and MFR vs. RT relationships with the y-intercepts and slopes calculated. An exponential model fitted to the MFR vs. $MUAP_{AMP}$ relationships with the *A* (scale factor) and *B* (rate of decay) terms calculated. In addition, non-voluntary twitch torques at rest (TQ_{REST}), normalized EMG amplitude ($N\text{-}EMG_{RMS}$) and percent voluntary activation (%VA) during the 70% MVC, and muscle cross-sectional area (CSA) of the VL were assessed pre- and post-treatment. Peak torque, TQ_{REST}, CSA, and the slopes of the $MUAP_{AMP}$ vs. RT relationships increased pre- to post-resistance training. The increased slopes indicated hypertrophy of the higher-threshold MUs post-resistance training. There were no changes in the MFR vs. RT relationships and the less negative *B* terms from the MFR vs. $MUAP_{AMP}$ relationships were a function of changing $MUAP_{AMPS}$. Lower $N\text{-}EMG_{RMS}$ and %VA, with no change in MFRs suggests reduced MU recruitment to sustain the same absolute torque post-resistance training. The findings suggest that resistance training increased sizes and twitch forces of MUs, but did not alter firing rates at steady torque. Hypertrophy reduced the number of MUs needed to sustain the same torque post-resistance training.

4.1 INTRODUCTION

It is well documented that resistance training increases strength and muscle hypertrophy (1). Although muscle hypertrophy partially accounts for the increases in maximal strength, early increases in strength have been attributed to neural adaptations (2, 3). The most commonly reported neuromuscular adaptations to resistance training are increased surface electromyography (EMG) amplitude and percent voluntary activation during maximal voluntary contractions (MVCs) (2-6). EMG amplitude is influenced by the number of active motor units (MUs), amplitudes of the action potentials (AP) (7), and firing rates (2). Therefore, EMG amplitude is considered a global measurement of muscle activation, particularly when normalized to peak EMG amplitude from a MVC (7). Percent voluntary activation, via the interpolated twitch technique, provides a crude estimate of MU recruitment. Nonetheless, these findings suggest resistance training elicits adaptations at the MU level, potentially altering MU control mechanisms during maximal and submaximal force production.

There is limited information regarding the influence of resistance training on MU firing rates during submaximal contractions. Following short-term resistance training interventions (3-10 weeks), researchers have reported no change (6, 8-11) or increases in firing rates (12). Methodological differences in the analysis of MU firing rates likely explain the conflicting results. Beck et al. (8) and Stock and Thompson (11) analyzed firing rates relative to recruitment threshold (RT), whereas, the Vila-Cha et al. (12) calculated a mean firing rate from MU data averaged across subjects and did not account for RTs. The firing rates of MUs are not homogeneous within the motoneuron pool, but instead demonstrate a strong negative relationship with RT (13-15) due to earlier recruited lower-threshold MUs having greater firing rates than later recruited higher-threshold MUs. Without accounting for RTs of recorded MUs, differences

in the number of recorded higher- vs. lower-threshold MUs will bias the results. In addition, Trevino et al. (16) reported variability in firing rates amongst individuals as a function of the myosin heavy chain area of the muscle, indicating that firing rates should be analyzed on a subject-by-subject basis. Therefore, further work is needed to better understand the influence of resistance training on MU firing rates.

Previously, motoneuron size (17) and the diameter of the muscle fibers that comprise the MU have been correlated with the amplitude of the MU action potential (MUAP) (18). Thus, analysis of MUAP amplitude can provide valuable insight on the size of MUs active during a contraction. There is strong positive relationship when MUAP amplitudes are expressed relative to RTs (14, 19-21) in accordance with Henneman's size principle (22). In addition, an increase in the slope of the MUAP amplitude vs. RT relationship provides a measure of muscle fiber hypertrophy. For example, resistance-training induced increases in muscle cross-sectional area (CSA) have been correlated with the changes in the slopes of the MUAP amplitude vs. RT relationships (19). Furthermore, the twitch force of a MU is associated with the MUAP amplitude (23, 24) and, therefore, MUs with larger MUAP amplitudes have greater twitch forces. It is speculated that muscle fiber hypertrophy (*MU hypertrophy*), as measured with MUAP amplitudes, could increase MU twitch forces and, thus, reduce the number of MUs required to sustain a given torque.

The majority of previous resistance training investigations have analyzed MU firing rates or other neuromuscular parameters at a percentage of the post-treatment MVC (6, 8-10, 12). Resistance training, however, is often utilized to improve performance or function in tasks in which the absolute force demands are not relative to maximal strength, such as carrying groceries or other activities of daily living. Thus, investigations examining the effects of

resistance training on properties of MUs should include contractions at the same tasks pre- and post-resistance training and relative to the higher MVC post-resistance training for a better understanding of potential adaptations to MU properties.

Therefore, the primary purpose of this investigation was to determine the effects of a short-term resistance training program (8 weeks) on MU firing rates and AP amplitudes in relation to recruitment thresholds of vastus lateralis (VL). Potential adaptations will be examined during isometric contractions performed at the same absolute torque pre- and post-resistance training and at the new relative MVC torque post-resistance training. It is hypothesized that the AP amplitudes of the higher-threshold MUs will increase and result in fewer MUs necessary to match the same absolute torque level post-resistance training. In addition, firing rates in relation to RT will be unaltered as previously reported (8, 11), however, firing rate patterns will change when expressed as a function of AP amplitudes because of MU hypertrophy. Non-voluntary twitch torques, percent voluntary activation, and normalized EMG amplitude will be measured to identify possible changes in twitch torques, muscle activation, and recruitment patterns during the submaximal isometric muscle actions. It is hypothesized that MU twitch torques will increase post-resistance training and normalized EMG amplitude and percent voluntary activation will decrease at the same absolute torque post-resistance training. Finally, MVC peak torque and EMG amplitude and muscle CSA will be measured pre- and post-resistance training.

4.2 METHODS

Subjects

Twenty-six healthy, college-aged men completed this investigation. Sixteen subjects completed the resistance training (TR) program (age: 20.7 ± 1.9 years; height: 178.4 ± 7.8 cm; weight: 75.9 ± 9.4 kg) and ten served as controls (CON) (age: 19.1 ± 2.3 years; height: 181.4 ± 6.9 cm; weight: 86.8 ± 20.4 kg) group. Subjects were physically active but had not participated in lower body resistance training in the previous 6 months. Prior to participation, all subjects completed a health history questionnaire and reported no neuromuscular conditions or musculoskeletal injuries that could impact the results of this investigation. Informed consent was obtained from all subjects. The institutional review board for human subjects approved this investigation.

Experimental Approach

To determine neuromuscular adaptations to resistance training, TR and CON were tested before and after an eight-week treatment period. Prior to each experimental testing visit, all subjects completed a familiarization visit, at which isometric MVCs and submaximal isometric trapezoid muscle actions of the knee extensors at 70% MVC were practiced. During each familiarization visit, ultrasound images were collected for CSA and subcutaneous fat thickness (FT) measurements of the VL. During each experimental testing visit, subjects performed MVCs and submaximal isometric trapezoid muscle actions at 70% MVC. MVCs were used to evaluate maximal voluntary strength and to determine the torque used for the submaximal contractions for pre- (PRE) and post-treatment. For post-treatment testing, the subjects completed muscle actions

at the new 70% MVC ($POST_{REL}$) and the same absolute torque as pre-treatment ($POST_{ABS}$). As a measure of non-voluntary strength, femoral nerve stimulation was administered to measure resting peak twitch torque. In addition, femoral nerve stimulation was used to administer superimposed and potentiated non-voluntary twitches during PRE and $POST_{ABS}$ contractions to quantify percent voluntary activation (%VA). Surface EMG signals were collected from the VL to measure peak EMG amplitude during MVCs and normalized EMG amplitude during the 70% MVCs. Surface EMG signals recorded during the PRE, $POST_{REL}$, and $POST_{ABS}$ contractions were decomposed to yield a mean firing rate (MFR), MUAP amplitude, and RT for each MU. The overall study design is presented in Figure 1.

Resistance Training Program

Subjects in the training group completed three lower body resistance-training sessions per week for 8 weeks. At each visit, subjects performed 4 lower body exercises, including complex multi-joint movements and single-joint isolation exercises. The lower body exercises included: back squats, front squats, Romanian deadlifts, leg extensions, leg presses, glute bridges, step ups, hamstring curls, and reverse hyperextensions. The training program was based on a linear periodization model with volume decreasing and intensity increasing over the course of the program. Subjects performed 3 sets of 12 repetitions during weeks 1-3, 3 sets of 8 repetitions during weeks 4-6 and 4 sets of 5 repetitions during weeks 7-8. The intensity for each set was based on the repetitions in reserve scale previously reported by Helms et al. (25). After each set the subjects reported their repetitions in reserve rating, and that rating along with the judgment of the trainer was used to determine the intensity of the following set. All training sessions were

overseen by National Strength and Conditioning Association Certified Strength and Conditioning Specialists. Subjects in the TR group completed all exercise sessions, whereas, subjects in the CON group did not perform any resistance training during the investigation.

Isometric Strength Testing

Subjects performed isometric strength testing of the right knee extensors with the leg positioned at 90° flexion. Testing was performed on a Biodex System 3 isometric dynamometer with subjects seated and restraining straps positioned over the pelvis, trunk and contralateral thigh. The lateral condyle of the femur was aligned with the input axis of the dynamometer. The isometric strength was measured as the torque signal from the dynamometer.

At each testing visit, subjects completed two three-second maximal voluntary contractions of the leg extensors with strong verbal encouragement. The highest observed MVC torque was used to determine the torque level for the subsequent submaximal contractions. At the pre-treatment testing visit subjects performed isometric trapezoidal muscle actions at 70% of MVC (PRE). At the post-treatment visit, subjects performed a muscle action at 70% of pre-treatment MVC to examine MU properties at the same absolute force ($POST_{ABS}$) and at 70% of post-treatment MVCs to examine MU properties at the same relative force ($POST_{REL}$) (Figure 1). Between each muscle action, subjects were given five to seven minutes of rest. Each isometric trapezoidal muscle (Figure 2) consisted of a linear torque increase, a 10s plateau and a linear torque decrease. During muscle actions, torque increased and decreased at a rate of 20% MVC/s. Subjects were provided with a visual template of the isometric trapezoidal muscle and real-time

torque feedback. A second attempt was provided when subjects were unable to adhere to the template during the initial attempt.

Electromyographic Recording & Decomposition

During the isometric trapezoidal muscle actions, surface EMG signals were collected from the VL via a Delsys 5-pin surface sensor array. The array consisted of five 0.5mm pins arranged in a 5x5 mm square with the fifth pin positioned in the center. The sensor was positioned at approximately 60% of the distance between the greater trochanter and the lateral condyle of the femur via hypoallergenic tape. A reference electrode was placed over the left patella. Prior to the sensor and reference electrode placement, the sites were shaved, dry skin cells were removed via repeated application of adhesive tape, and the sites was sterilized with alcohol. The signals from the electrode's four pairs of the pins were differentially amplified and filtered at a band-pass of 20-450 Hz. The EMG signals were sampled at 20 kHz and stored for subsequent decomposition and analysis. Using the previously detailed Precision III algorithm, the four channels of raw surface EMG data collected by the 5-pin sensor array were decomposed into their constituent MU action potential trains. The accuracy of the decomposed action potential trains were tested via the reconstruct-and-test procedure and only MUs that demonstrated $\geq$ 90% accuracy were included in the subsequent analyses (26).

The action potential trains were low-pass filtered with a unit area Hanning window (2s duration) to compute the firing rates of each MU. Analyses yielded four parameters per MU: the recruitment threshold (RT), mean firing rate (MFR), MUAP amplitude ($MUAP_{AMP}$) and the MUAP duration ($MUAP_{DUR}$). The RT was calculated as the average torque during the 0.01

second epoch following the first firing of the MU and was expressed relative to the MVC torque. The MFR was calculated as the average firing rate during the steady torque region of the isometric trapezoidal muscle action. The $MUAP_{AMP}$ was calculated as the average peak-to-peak amplitude of the each of the four unique action potential waveform templates in accordance with previously reported methods (14, 19, 21). The $MUAP_{DUR}$ was calculated as the average peak-to-peak duration of the four unique action potential waveform templates.

EMG Amplitude

Channel 1 of the surface sensor array's 4 bipolar EMG channels was selected for EMG amplitude analysis. The EMG signals were bandpass filtered (zero phase fourth-order Butterworth filter) at 10-500 Hz. Peak EMG amplitudes ($P\text{-}EMG_{RMS}$) were calculated as the average root mean square (RMS) value during the highest 0.25 s torque epoch observed during the pre- and post-treatment MVCs. In addition, the average EMG_{RMS} during the steady torque regions of the PRE, $POST_{ABS}$ and $POST_{REL}$ isometric trapezoidal muscle actions were calculated and normalized ($N\text{-}EMG_{RMS}$) to $P\text{-}EMG_{RMS}$. EMG_{RMS} during PRE was normalized to pre-treatment $P\text{-}EMG_{RMS,}$ whereas, the EMG_{RMS} during $POST_{ABS}$ and $POST_{REL}$ was normalized to post-treatment $P\text{-}EMG_{RMS}$.

Resting Twitch Torque, Twitch Potentiation, and Percent Voluntary Activation

To analyze resting twitch torque (TQ_{REST}) and percent voluntary activation (%VA), transcutaneous electrical stimuli were delivered to the femoral nerve via a high-voltage, constant-current stimulator (Digitimer DS7AH, Herthfordshire, UK). Prior to data collection, the

optimal stimulus location was determined using a bipolar probe with soaked felt tips with single stimuli administered at a relatively low current (amperage = 80 mA). After determining and marking the optimal location, further stimuli were administered via adhesive electrodes. To determine the amperage necessary to elicit a maximal contraction, stimuli were delivered beginning at 80 mA and increasing 10 – 20 mA until the elicited twitch torque plateaued for three straight stimuli. The supramaximal stimulus used to evoke resting, potentiated, and superimposed twitches was 120% of the maximal amperage that elicited the highest peak torque. Peak twitch torques were calculated as the average of the highest 0.05 s of torque. The TQ_{REST} was the calculated peak twitch torque from the pre-contraction twitch.

Superimposed twitch torque was measured during the steady torque region of isometric trapezoidal muscle actions at the same intensity the pre- (PRE) and post-training ($POST_{ABS}$) contractions. Subjects traced the trapezoidal template up to the steady torque region, after which, a supramaximal stimulus was delivered to the femoral nerve. The superimposed twitch torque was measured as the peak torque elicited by the stimulus minus the torque immediately preceding the stimulus with %VA calculated via the following equation: %VA = 1 – (superimposed twitch torque ÷ potentiated twitch torque) x 100. Due to an inability to elicit a consistent twitch as monitored via TQ_{REST}, one TR and CON subject were excluded from the TQ_{REST} and %VA analyses.

Ultrasound Imaging

To measure muscle CSA and FT, transverse ultrasound images of the right VL were collected using a NextGen LOGIQ c ultrasound console (GE Healthcare UK, Ltd., Chalfont,

Buckinghamshire, UK) with a multi-frequency linear array transducer (Model 12L-RS; 5-13 MHz; 38.4 mm field-of-view). Images were collected using brightness mode imaging with the equipment settings (depth: 6.0 cm; gain: 49 dB; frequency: 10 MHz) constant across all subjects. For two subjects, the 6.0 cm depth was insufficient to fully capture the VL and, thus, CSA and FT were not calculated for these subjects. Prior to imaging, subjects rested supine for 10 minutes to allow fluid shifts to occur. After the rest period, transverse, panoramic images were collected at 50% of the distance from the anterior superior iliac spine to the superior patella. During image capture, a custom-made probe support composed of high-density foam padding was positioned perpendicular to the longitudinal axis of the thigh to ensure ultrasound probe movement in the transverse plane. Generous amounts of ultrasound gel were used to ensure minimal pressure on the skin. CSA and FT were calculated using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Images were scaled from pixels to cm using the straight-line function. To calculate CSA the periphery of the VL was outlined using the polygon function with care taken to exclude the surrounding fascia. FT was calculated as the straight-line distance from the bottom of the cutaneous layer to the top of the muscle fascia.

Statistical Analysis

For MU data, $MUAP_{AMP}$ was linearly regressed against RT for each contraction to yield a slope and y-intercept value. Y-intercept values were not statistically analyzed due to limited observation of MUs with RTs < 20% MVC in conjunction with rapid increases in $MUAP_{AMPS}$ of the higher-threshold MUs, which resulted in negative y-intercept values in most instances (58 of 72 relationships). Therefore, to determine if the $MUAP_{AMP}$ of the lower-threshold MUs recorded

during the 70% MVC was altered by resistance training, the slope and y-intercept values of the individual $MUAP_{AMP}$ vs. RT relationships were used to calculate the estimated AP_{AMP} of a MU with a RT of 30% MVC for each individual at PRE, $POST_{ABS}$ and $POST_{REL}$ (Figure 2). The slopes of the relationships and estimated $MUAP_{AMPS}$ at 30% MVC were used for statistical analysis. The RT of 30% MVC was chosen as that was close to the average RT of the lowest-threshold MU observed for each contraction (PRE, $POST_{ABS}$, and $POST_{REL}$). For the MFR vs. RT relationship, MFRs were linearly regressed against RT for each individual to yield a slope and y-intercept value for statistical analysis (Figure 2). In addition, an exponential model was applied to each individual's MFR vs. $MUAP_{AMP}$ relationship to yield a *B* term and *A* term for statistical analysis (Figure 2). In the MFR vs. $MUAP_{AMP}$ model, $MFR=Ae^{B(MUAPAMP)}$, where the *A* term is the MFR scale factor, *e* is the natural constant, and the *B* term represents the rate of decay of MFR with increments in $MUAP_{AMP}$.

$MUAP_{DUR}$ has been reported as a measure of MU depth (14, 27) and, thus, $MUAP_{DUR}$ was linearly regressed against RT to determine if the depth of the observed MUs changed along the RT spectrum. However, similar to our previous work and others (14, 21, 28), few of these relationships were significant (14 of 78 relationships) and the slopes were small (0.101 – 0.042 ms/%MVC). Therefore, the depth of the MUs did not change as a function of RT and did not alter the interpretation of the $MUAP_{AMPS}$ (14, 21, 28). Instead, the average $MUAP_{DUR}$ of the observed MUs for each contraction was calculated and used for statistical analysis.

Potential changes in maximal peak torque, $P\text{-}EMG_{RMS}$, CSA, FT, $TQ_{REST,}$ and %VA were examined via separate two-way mixed factorial repeated measure ANOVAs [Group (TR vs. CON) x Time (PRE vs. POST)]. Eight separate two-way mixed factorial repeated measure ANOVAs [Group (TR vs. CON) x Contraction (PRE vs. $POST_{ABS}$ vs. $POST_{REL}$)] were used to

analyze possible differences in N-EMG_{RMS}, slopes from the $MUAP_{AMP}$ vs. RT relationships, the estimated $MUAP_{AMPS}$ at 30% MVC, slopes and y-intercepts of the MFR vs. RT relationships, *B* and *A* terms from the MFR vs. $MUAP_{AMP}$ relationships, and the average $MUAP_{DURS}$. When appropriate, follow-up analyses were performed using one-way repeated measures ANOVAs, paired and independent samples t-tests with Bonferroni corrections. In addition, various correlations were performed on data of interest. Alpha was set at $p \leq 0.05$. All statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, NY).

4.3 RESULTS

Strength and Ultrasound Data

For peak torque, there was a significant two-way interaction ($p = 0.001$). There was a significant increase in peak torque pre- to post-treatment for the TR (PRE: 204.6 ± 34.9 Nm; POST: 239.8 ± 36.3 Nm; $p < 0.001$), whereas, there was no significant change for the CON (PRE: 195.6 ± 49.2 Nm; POST: 199.2 ± 42.9 N ▪m; $p = 0.453$). There was no significant difference between groups for the pre-treatment peak torques ($p = 0.589$), however, there was a significant difference post-treatment ($p = 0.016$).

For P-EMG_{RMS}, there was a significant two-way interaction ($p = 0.039$). There was a significant increase from pre- to post-treatment for the TR (PRE: 108.0 ± 55.6 mV; POST 139.2 ± 52.4 mV; $p = 0.014$), but no change for the CON (PRE: 78.3 ± 23.2 mV; POST 77.1 ± 26.4 mV; $p = 0.819$). There were no significant pre-treatment differences between groups ($p = 0.124$), however, TR demonstrated significantly greater P-EMG_{RMS} than CON post-treatment ($p = 0.001$).

For TQ_{REST}, there was a significant two-way interaction ($p = 0.002$). There was a significant increase in pre- to post-treatment for the TR (PRE: 44.6 ± 7.4 Nm; POST: 48.3 ± 11.2 Nm; $p = 0.014$), whereas, there was a significant decrease for the CON (PRE: 45.4 ± 8.0 Nm; POST: 42.5 ± 5.6 Nm; $p = 0.014$). There were no differences between groups at pre- ($p = 0.796$) or post-treatment ($p = 0.162$).

For CSA, there was a significant two-way interaction ($p = 0.001$). There was a significant increase pre- to post-treatment for the TR (PRE: 44.6 ± 28.3 cm^2; POST: 34.0 ± 5.0 cm^2; $p = 0.014$), however, was unchanged for the CON ($p = 0.453$; PRE: 30.0 ± 6.4 cm^2; POST: 30.9 ± 5.1 cm^2; $p = 0.453$). There were no significant differences between groups for pre- ($p = 0.596$) or post-treatment ($p = 0.163$).

For FT, there was no two-way interaction ($p = 0.814$) or main effects for group ($p = 0.716$) or time ($p = 0.359$). There was no change for the TR (PRE: 0.46 ± 0.24 cm; POST: 0.43 ± 0.21 cm) or CON (PRE: 0.42 ± 0.24 cm; POST: 0.40 ± 0.23 cm). Therefore, FT was not a confounding factor when interpreting possible changes in EMG_{RMS} and $MUAP_{AMPS}$ as a function of resistance training.

N-EMG_{RMS} and VA Data

For N-EMG_{RMS}, there was a significant two-way interaction ($p = 0.005$). The one-way ANOVAs for contraction was significant for TR ($p < 0.001$), but not for CON (PRE: 75.8 ± 13.0%; $POST_{ABS}$: 77.1 ± 14.1%; $POST_{REL}$: 81.0 ± 11.1%; $p = 0.444$). For the TR, $POST_{ABS}$ (60.4 ± 16.8%) was lower than PRE (76.9 ± 14.2%) ($p < 0.001$) and $POST_{REL}$ (79.4 ± 21.9%) ($p < 0.001$), however, there were no significant difference between PRE and $POST_{REL}$ ($p = 0.999$).

There were no significant differences between groups at PRE ($p = 0.853$) and $POST_{REL}$ ($p = 0.807$), but there was a significant difference between groups at $POST_{ABS}$ ($p = 0.015$).

For %VA, there was a significant two-way interaction ($p = 0.021$). There was a significant decrease pre- to post-treatment for the TR (PRE: 84.0 ± 7.3%; POST: 79.8 ± 7.5 %; $p = 0.033$), but no change was observed for the CON (PRE: 79.4 ± 9.8%; POST: 82.6 ± 8.0%; $p = 0.201$). There were no significant differences between groups at pre- ($p = 0.200$) or post-treatment ($p = 0.392$).

MU Data

A total of 1429 MUs were recorded, with similar average MU counts observed in each of the three contractions for the TR (PRE: 17.1 ± 3.7; $POST_{ABS}$: 19.4 ± 5.9; $POST_{REL}$: 18.0 ± 6.0) and CON (PRE: 19.1 ± 4.0; $POST_{ABS}$: 18.8 ± 7.3; $POST_{REL}$: 17.7 ± 5.9). The RT ranges of the recorded MUs for the TR were 24.2 – 61.9%, 26.5 – 63.2% and 29.8 – 62.6% for the PRE, $POST_{ABS}$ and $POST_{REL}$, respectively. The RT ranges of the recorded MUs for the CON were 23.0 – 62.0%, 23.2 – 55.7% and 26.3 – 57.5% at PRE, $POST_{ABS}$ and $POST_{REL}$, respectively. The $MUAP_{AMP}$ ($r = 0.805 \pm 0.098$) and MFR ($r = -0.925 \pm 0.055$) vs. RT and MFR vs. $MUAP_{AMP}$ ($r = 0.848 \pm 0.081$) relationships were significant for each contraction.

For the slopes of the $MUAP_{AMP}$ vs. RT relationships, there was a significant two-way interaction ($p = 0.032$). The one-way ANOVA for contraction was significant for TR ($p = 0.001$), but not for CON ($p = 0.335$). For the TR, the slopes were greater for the $POST_{REL}$ than PRE ($p = 0.007$) and $POST_{ABS}$ ($p = 0.001$), however, there was no significant difference between PRE and $POST_{ABS}$ ($p = 0.999$) (Figure 3). There were significant differences between groups for $POST_{REL}$

($p = 0.002$) and $POST_{ABS}$ ($p = 0.015$), but not for PRE ($p = 0.114$). For estimated $MUAP_{AMPS}$ of MUs recruited at 30% MVC, there was no two-way interaction ($p = 0.838$) or main effects for contraction ($p = 0.821$) and group ($p = 0.345$) (Figure 3). There was no significant correlation ($p = 0.413$, $r = -0.220$) between the change in CSAs and the change in the slopes from the $MUAP_{AMP}$ vs. RT relationships for the TR.

For the MFR vs. RT relationships, there were no two-way interactions ($p = 0.831$, $p = 0.786$) or main effects for group ($p = 0.403$, $p = 0.420$) or contraction ($p = 0.386$, $p = 0.157$) for the slopes and y-intercepts (Figure 4).

For the MFR vs. $MUAP_{AMP}$ relationships, there was a two-way interaction ($p = 0.040$) for the *B* terms. The one-way ANOVAs for contraction was significant for TR ($p = 0.001$), but not for CON ($p = 0.575$). The *B* terms where less negative for the $POST_{REL}$ than PRE ($p = 0.007$) and $POST_{ABS}$ ($p = 0.050$), however, there was no significant difference between PRE and $POST_{ABS}$ ($p = 0.295$) (Figure 5). There were significant differences between groups for $POST_{REL}$ ($p = 0.003$) and $POST_{ABS}$ ($p = 0.044$), but not for PRE ($p = 0.116$). For the *A* terms, there was no significant two-way interaction ($p = 0.472$) or main effects for group ($p = 0.625$) and contraction ($p = 0.756$).

For $MUAP_{DURS}$, there was no two-way interaction ($p = 0.647$) or main effects for group ($p = 0.382$) or contraction ($p = 0.234$). There was no change in average $MUAP_{DUR}$ in the TR (PRE: 5.60 ± 0.46 ms; $POST_{ABS}$: 5.82 ± 0.61 ms; $POST_{REL}$: 5.85 ± 0.33 ms) or CON (PRE: 5.56 ± 0.60 ms; $POST_{ABS}$: 5.65 ± 0.58 ms; $POST_{REL}$: 5.61 ± 0.43 ms). Thus, the average depths of recorded MUs were consistent between groups and contractions and were not a confounding factor when interpreting $MUAP_{AMPS}$ (14, 27).

4.4 DISCUSSION

In this investigation, eight weeks of lower body resistance training significantly increased maximal voluntary and non-voluntary leg extensor torque in the presence of hypertrophy of the VL. The slopes from the $MUAP_{AMP}$ vs. RT relationship indicated that the higher-threshold MUs were larger following resistance training. A novel finding of the present study was that MFRs in relation to RT were not altered, whereas, the changes in the MFR vs. $MUAP_{AMP}$ relationships were a function of enlarged $MUAP_{AMPS}$ rather than changes in MFRs. In addition, lower muscle activation was required to sustain the same absolute torque task post-resistance training. Therefore, 8 weeks of resistance training increased the diameters of muscles fibers that comprise higher-threshold MUs but did not alter the firing rates at the targeted torques.

The eight-week resistance-training program elicited significant muscle hypertrophy as indicated by an average increase CSA of 17.9%. Previous reports of CSA increases in untrained individuals following short-term resistance training interventions have ranged from 10 – 17% (19, 29, 30). The observed hypertrophy in the present study is slightly larger than, but in accordance with previous investigations. The slightly larger hypertrophy in the present study may be due to differences in exercise choice, training volume or training intervention duration or the usage of repetitions in reserve scale (25).

$MUAP_{AMP}$ has been previously correlated with the size of the motoneuron (17). Thus, the strong positive correlations between $MUAP_{AMPS}$ and RTs observed in this and previous investigations (14, 19-21) support the orderly recruitment of MUs according to motoneuron size (22). In contrast, Del Vecchio et al. (31) reported weak or no relationships between the MUAP root mean square (RMS) in relation to RTs. The difference between studies is likely due to the methods used to obtain and calculate the amplitude of the MUAPs. Del Vecchio et al. (31) used a

decomposition method with a high-density EMG grid electrode that yields the firing instances of MUs. The calculation of the MUAP RMS requires a spike triggered averaging technique that takes in account the EMG immediately following the firing instances in longitudinal direction of the electrode to obtain the amplitude of the AP. In the present study, the decomposition method yields four unique MUAP waveforms from a 5-pin sensor with the peak-to-peak amplitudes averaged across the four channels used to regress against RTs. In addition, Del Vecchio et al. (31) analyzed the MUAP RMS vs. RT relationships using composite data from contractions of different intensities, unlike the present investigation where the $MUAP_{AMP}$ vs. RT relationships were analyzed from a single contraction. Of note, Martinez-Valdes et al. (7) used similar methods as Del Vecchio et al. (31), however, the authors incorporated an EMG normalization procedure that resulted in a significantly positive composite (included all subjects and contractions) MUAP RMS vs. RT relationship that more closely aligns with findings in the present study. Nonetheless, caution is warranted when comparing the slopes from the $MUAP_{AMPS}$ vs. RT relationships in the present study with the information provided by the MUAP RMS vs. RT relationships presented in other studies (7, 31) as these two methods of obtaining AP amplitudes and subsequent statistical procedures are fundamentally different.

The slopes of the $MUAP_{AMP}$ vs. RT relationships provide an indication of MU sizes across the force spectrum and is sensitive to changes in resistance training induced MU hypertrophy in the VL (19) and age-related atrophy of the first dorsal interosseous (20). Considering only the $POST_{REL}$ vs. PRE contractions where muscle activation ($N\text{-}EMG_{RMS}$: 76.3 ± 13.6% vs. 76.6 ± 14.4%) was equivocal, the increase in the slopes from the $MUAP_{AMP}$ vs. RT relationships ($POST_{REL}$ vs. PRE) with no changes in the $MUAP_{AMPS}$ of the recorded lowest-threshold MUs (RT = 30% MVC) suggests that hypertrophy was present only for the later

recruited, higher-threshold MUs. Similar to Pope et al. (19), the increases in AP_{AMPS} was isolated to MUs with RTs > 35% MVC. Unlike Pope et al. (19), the absolute increase in CSAs were not significantly correlated with the absolute increase in the slopes from the $MUAP_{AMP}$ vs. RT relationships. The lack of correlation in the present investigation is likely the result of differences in the observed RT ranges and the contraction intensity tested (70% MVC). Specifically, Pope et al. (19), observed larger RT ranges (Pre: 13.7 – 64.2%; Post: 20.1 – 67.6%) and included a 90% MVC, which would have recruited a larger portion of the MU pool. Nonetheless, the increase in the slopes of the $MUAP_{AMP}$ vs. RT relationships with no changes in the AP_{AMPS} of the recorded lowest-threshold MUs suggests that the resistance training-related increase in muscle CSA was due to hypertrophy of the higher-threshold MUs.

The difference in hypertrophy between lower- and higher-threshold MUs was likely due to the muscle fiber type characteristics. Although muscle fiber co-expression of type I and type II fiber characteristics results in a continuum of twitch force properties (32), lower-threshold MUs commonly exhibit twitch force properties associated with type I muscle fibers, whereas, higher-threshold MUs exhibit twitch force properties more associated with type II muscle fibers (33-35). Therefore, the greater hypertrophy observed in the higher-threshold MUs was likely due to hypertrophy of muscle fibers that primarily express type II characteristics in accordance with previous reports of greater hypertrophy in type II muscle fibers than type I muscle fibers (29, 36). In addition, Colquhoun et al (37) recently reported a non-significant positive relationship between type II myosin heavy chain area and the slopes of the $MUAP_{AMP}$ vs. RT relationships in resistance trained men, providing further support that the greater slopes post-resistance training in the present study were due to hypertrophy of higher-threshold MUs that likely exhibited greater area of muscle fibers that primarily express type II characteristics.

Previously, research studies have reported a strong, positive relationship between MU twitch force and $MUAP_{AMP}$ (23, 24). Therefore, the observed increase in $MUAP_{AMPS}$ following resistance training suggests an increase in the maximal twitch forces of the higher-threshold MUs. Although individual MU twitch forces were not measured in the present study, resistance training did increase the TQ_{REST}. Thus, the increase in TQ_{REST} suggests that MU twitch forces were increased post-resistance training. The increased MU twitch forces were likely a significant contributor to the increase in maximal isometric strength post-resistance training.

An increase in twitch forces may have resulted in fewer MUs needed to sustain the same absolute targeted torque post-resistance training as indicated by the decrease in $N\text{-}EMG_{RMS}$ and %VA. $N\text{-}EMG_{RMS}$ is considered a global measure of muscle activation and cannot distinguish between MU recruitment and firing rates (38), whereas, %VA is considered a crude assessment of MU recruitment. Nonetheless, the decreases in %VA and $N\text{-}EMG_{RMS}$ with no changes in MFRs tentatively suggest that there was decreased MU recruitment at the same absolute torque post-resistance training. In agreement, Jenkins et al. (4) observed a similar decrease in the %VA at the same absolute force following a resistance training program. In addition, the similar slopes of the $MUAP_{AMP}$ vs. RT relationship despite lower $N\text{-}EMG_{RMS}$ and %VA during the PRE and $POST_{ABS}$ contractions would suggest hypertrophy of MUs was present. If hypertrophy of MUs was not present, the $MUAP_{AMPS}$ would be less during a contraction with fewer recruited MUs (lower $N\text{-}EMG_{RMS}$ and %VA) as observed between the slopes for the post-resistance training contractions ($POST_{ABS} < POST_{REL}$).

A strong negative relationship between MFR and RT was observed in all contractions, in accordance with the onion-skin control scheme (13-15), however, MFR vs. RT relationships were unchanged by the resistance training program. Therefore, the findings of the present study

indicated that there were no effects of resistance training on MU firing rates during submaximal contractions, which is in agreement with previous investigations (6, 8-11). In contrast, Vila-Cha et al. (12) reported an increase in MU firing rates during submaximal contractions following a resistance training intervention. Vila Cha et al. (12) reported an average of 4 MUs per contraction, with as few as 1 MU observed in a contraction, unlike the present study where 18 MUs were observed per contraction for each subject. Due to the low number of MUs observed, Vila Cha et al. (12) analyzed the average MU firing rates collapsed across subjects and did not account for RTs. Given that previous investigations have reported an increase in the number of lower-threshold MUs recorded following resistance training (39), the increase in MU firing rates reported by Vila Cha et al. (12) may best explained by a greater observation of lower-threshold MUs with higher MFRs rather than changes in the firing rates of motoneurons at steady torque. In addition, there is a considerable amount of variability in MFRs among subjects (16) and a few subjects could bias the results. Thus, when RTs are accounted for and data is analyzed on a subject-by-subject basis, resistance training may not influence MFRs at a targeted torque.

Although the relationships between MFRs and RTs did not change, TR possessed significantly less negative *B* terms of the MFR vs. $MUAP_{AMP}$ relationships, with no changes in the *A* terms following resistance training. Therefore, no differences in MFRs of the smaller MUs, but large differences in MFRs of the larger MUs existed following resistance training. The changes in MFR vs. $MUAP_{AMP}$ relationships were not likely due to changes in the MFRs at steady torque, but instead a result of the significant hypertrophy of the higher-threshold MUs. The smaller, low-threshold MUs demonstrated minimal hypertrophy, thus minimal changes in MFR in relation to $MUAP_{AMP}$ (no difference in *A* terms). In contrast, the larger higher-threshold MUs demonstrated significant hypertrophy and, thus, large changes in the MFR in relation to

$MUAP_{AMP}$ (Figure 6). In addition, the range of observed MFRs did not change following resistance training (PRE: 9.4 ± 1.2 – 22.4 ± 2.5 pps; $POST_{REL}$: 9.8 ± 1.5 – 23.1 ± 2.1 pps), whereas, there was a large increase in the range of observed $MUAP_{AMPS}$ (PRE: 0.100 ± 0.056 – 0.357 ± 0.190 mV; $POST_{REL}$: 0.132 ± 0.061 – 0.491 ± 0.270 mV) at the same relative muscle activation ($N\text{-}EMG_{RMS}$). Since the increase in $MUAP_{AMPS}$ was due to muscle fiber hypertrophy and firing rates are primarily a property of the motoneurons, the findings of the present study suggest that adaptations to short-term resistance training programs occur at the muscle fiber level rather than the motoneuron of the MU.

In agreement with previous investigations (2-4), a large increase in EMG_{RMS} (~29%) during the MVCs ($P\text{-}EMG_{RMS}$) was observed. However, given the lack of change in MFRs, it is unlikely that increases in $P\text{-}EMG_{RMS}$ were due to increases in MFRs. Of the three investigations that have analyzed MU firing rates during maximal isometric muscle actions pre- and post-resistance training, one has reported no change (6). The other two studies, Patten et al. (40) and Kamen and Knight (9) reported significant increases in maximal firing rates between two baseline visits prior to the training intervention, with no additional firing rate increases from the second baseline visit to the post-training visit. Thus, short-term resistance training does not appear to increase maximal MU firing rates. These previous findings, combined with the lack of change in the MFR vs. RT relationships suggest that the observed $P\text{-}EMG_{RMS}$ increase was not due to increases in maximal firing rates.

The increase in $P\text{-}EMG_{RMS}$ is also not likely due to large increases in MU recruitment. Previously, Pucci et al (6) and Knight et al (5) reported only small increases in %VA (2.7 and 1.7%) of the leg extensors. Similar to the present study, Pucci et al. (6) and Knight et al. (5) measured untrained college aged males. Therefore, %VA if administered during the MVC in the

present study would likely mimic the findings of Pucci et al. (2006) and Knight et al. (5). Recently, Martinez Valdez (7), reported that the amplitude of the EMG signal is largely explained by the size of the $MUAP_{AMPS}$ and, therefore, it is hypothesized that the routinely observed increase in maximal EMG_{RMS} following resistance training is predominantly due to an increase in $MUAP_{AMPS}$ as a result of muscle fiber hypertrophy.

In summary, the short-term resistance training program elicited significant increases in voluntary and non-voluntary strength (MVC torque and TQ_{REST}) and significant hypertrophy of the muscle and higher-threshold MUs. Resistance training did not alter MFRs relative to RT. There were changes the MFRs relative to $MUAP_{AMP}$, however, these changes were due to the hypertrophy of MUs rather than changes in the MFRs. In addition, resistance training-related increases in peak EMG_{RMS} were likely due to increases in $MUAP_{AMPS}$ and not in peak firing rates. The findings of this study tentatively indicate that acute adaptations to resistance training occur in skeletal muscle fibers and not of the motoneuron of the MUs. As a result of hypertrophy, individual MUs can produce more force and, thus, fewer MUs are required to sustain the same absolute torque post-resistance training. Of importance, resistance training may reduce the fatigue for a given submaximal load, which is relevant for aging individuals performing activities of daily living and tactical athletes in which load carriage is not relative to body weight.

4.5 FIGURES

Figure 1. The study design for the pre- and post-treatment strength testing visits. MVC = Maximal Voluntary Contraction. PRE = an isometric trapezoidal muscle action performed at 70% of the pre-training MVC. $POST_{ABS}$ = an isometric trapezoidal muscle action performed at 70% of the pre-training MVC. $POST_{REL}$ = an isometric trapezoidal muscle action performed at 70% of the post-training MVC. Vertical dash lines = femoral nerve stimulation to assess resting twitch torque, superimposed twitch torque, and potentiated twitch torque.

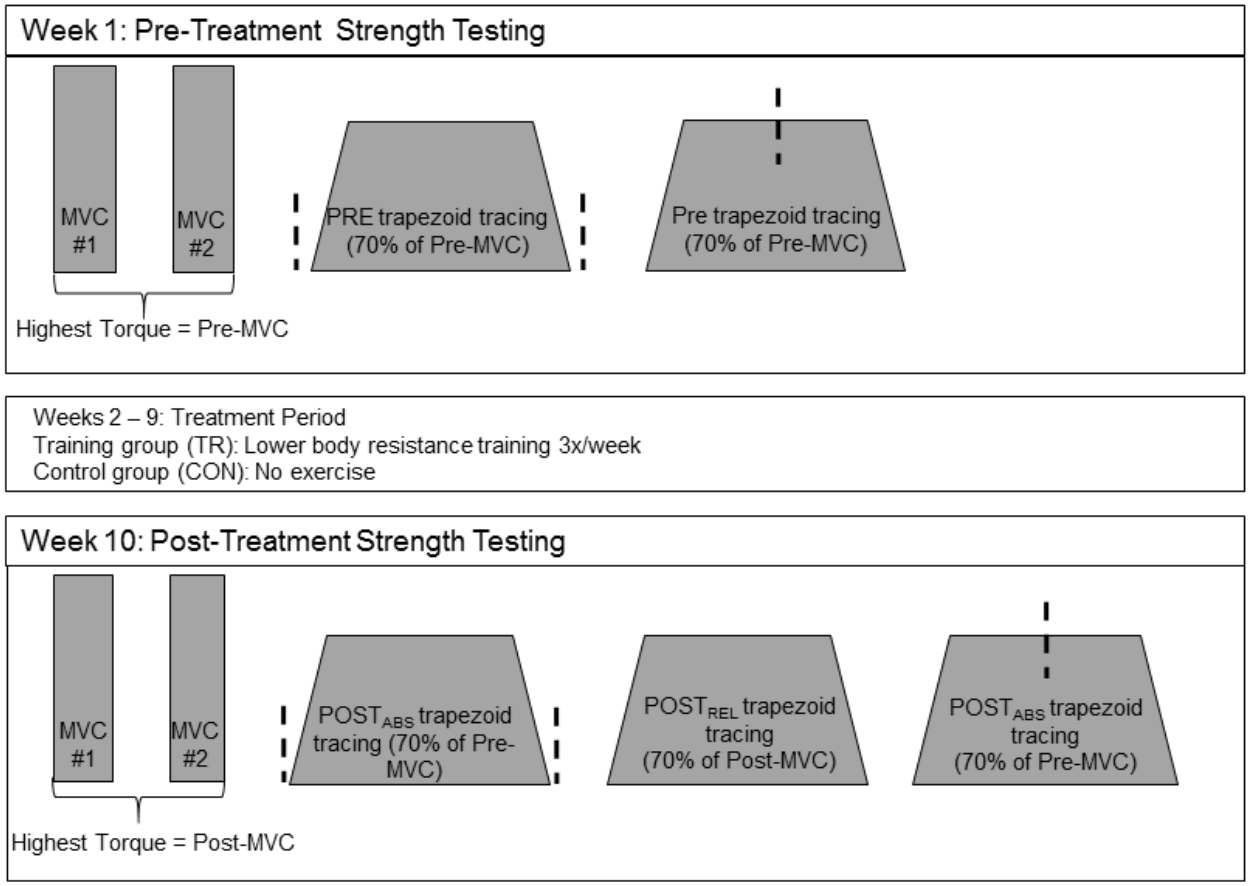


Figure 2. Top graph: The normalized torque and individual motor unit (MU) firing rates during an isometric trapezoidal muscle action performed at 70% maximal voluntary contraction (%MVC). The lower-threshold MU (MU4) maintains a higher firing rate than the higher-threshold MU (MU18) throughout the contraction. Middle graphs: The four unique MU action potential waveforms of the highlighted MUs used to calculate MU action potential amplitude ($MUAP_{AMP}$) and duration. Bottom graphs: The $MUAP_{AMP}$ vs. recruitment threshold, mean firing rate vs. recruitment threshold and mean firing rate vs. $MUAP_{AMP}$ relationships for the individual.

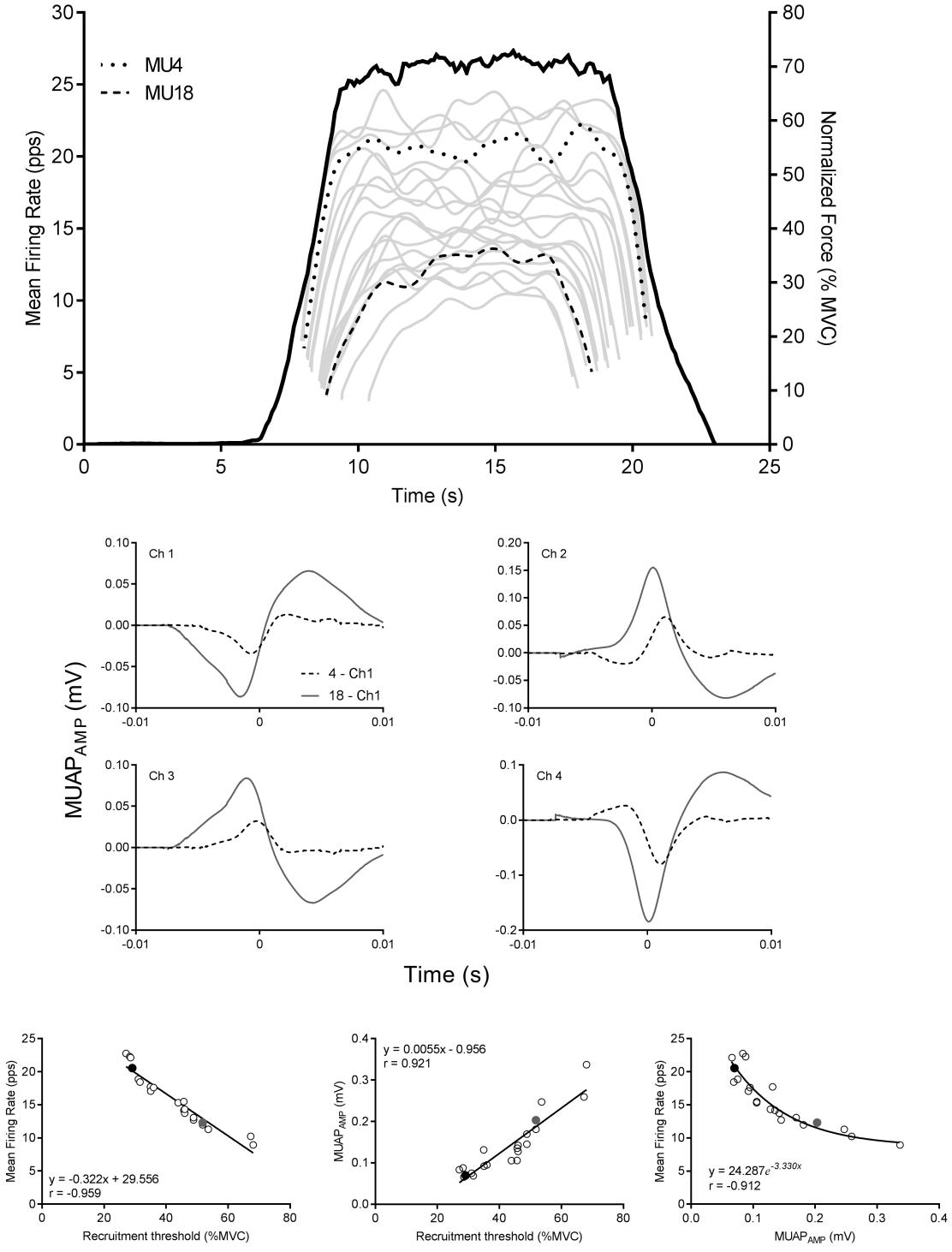


Figure 3. The mean (SD) slopes of the motor unit action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold (expressed as percent of maximal voluntary contraction [%MVC]) relationships (top left), mean (SD) estimated $MUAP_{AMPS}$ for MUs recruited at 30% MVC (top right), and composite $MUAP_{AMP}$ vs. recruitment threshold patterns for the resistance training group (TR) (bottom left) and control (CON) group (bottom right). The composite patterns are depicted for contractions performed pre-treatment at 70% of the pre-treatment MVC (PRE), post-treatment at 70% of the pre-treatment MVC ($POST_{ABS}$) and post-treatment at 70% of the post-treatment MVC ($POST_{REL}$). * Indicates that for TR, $POST_{REL}$ was greater than PRE or $POST_{ABS}$. † Indicates that TR was greater than CON for the respective contraction.

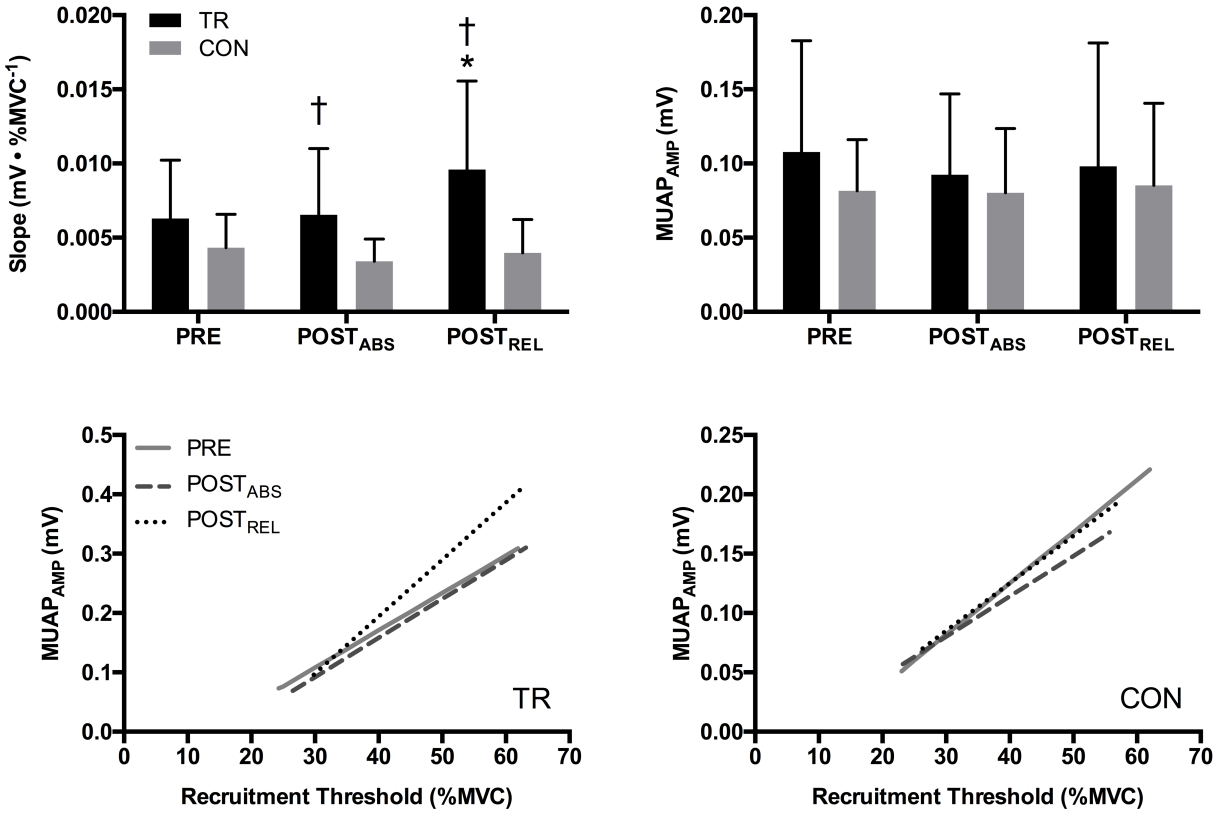


Figure 4. The mean (SD) slopes of the firing rate vs. recruitment threshold (expressed as percent of maximal voluntary contraction [%MVC]) relationship (top left), mean (SD) y-intercepts (top right), and composite patterns for the resistance-training group (TR) (bottom left) and control (CON) group (bottom right). The composite patterns are depicted for contractions performed pre-treatment at 70% of the pre-treatment MVC (PRE), post-treatment at 70% of the pre-treatment MVC ($POST_{ABS}$) and post-treatment at 70% of the post-treatment MVC ($POST_{REL}$).

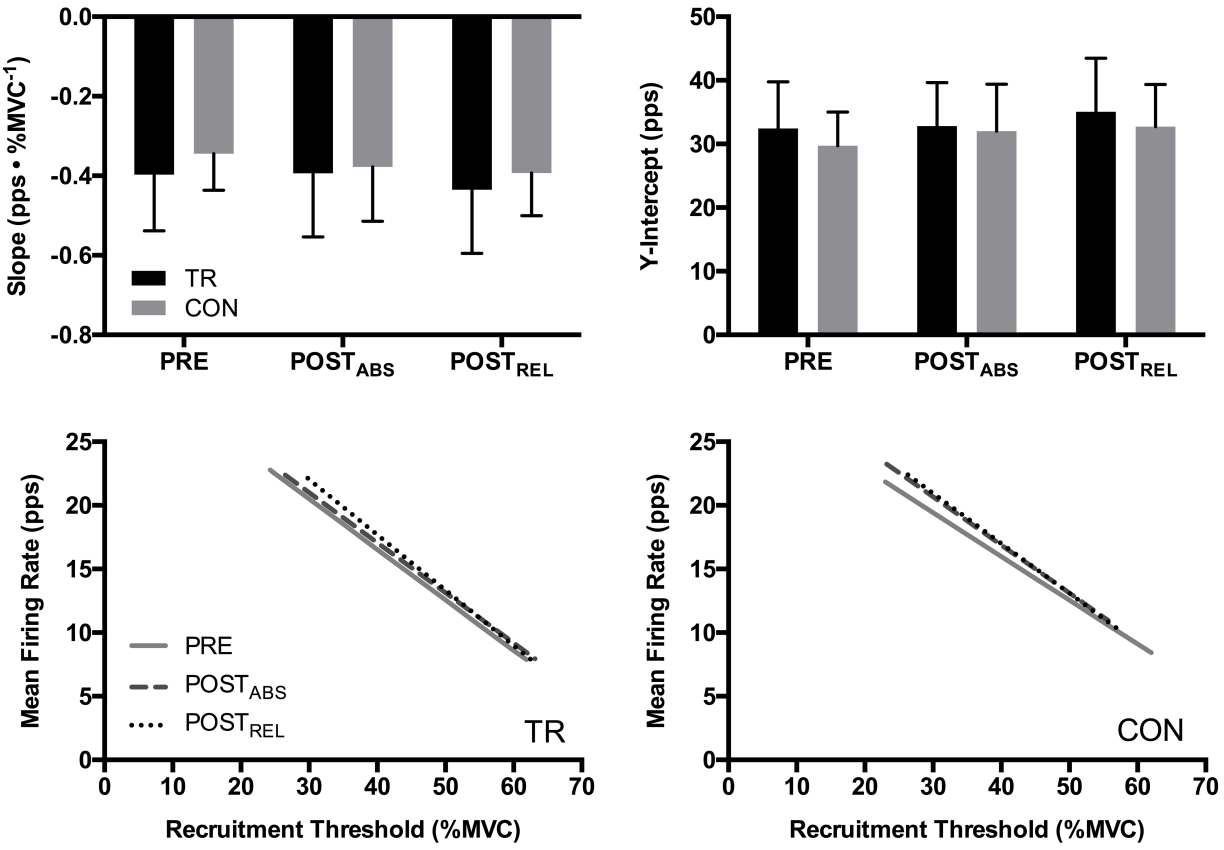


Figure 5. The mean (SD) *B* terms of the firing rate vs. motor unit action potential amplitude ($MUAP_{AMP}$) relationships (top left), mean (SD) *A* terms (top right) and composite patterns for the resistance-training group (TR) (bottom left), and control (CON) group (bottom right). The composite patterns are depicted for contractions performed pre-treatment at 70% of the pre-treatment MVC (PRE), post-treatment at 70% of the pre-treatment MVC ($POST_{ABS}$) and post-treatment at 70% of the post-treatment MVC ($POST_{REL}$). * Indicates that for TR, $POST_{REL}$ was greater than PRE or $POST_{ABS}$. † Indicates that TR was greater than CON for the respective contraction.

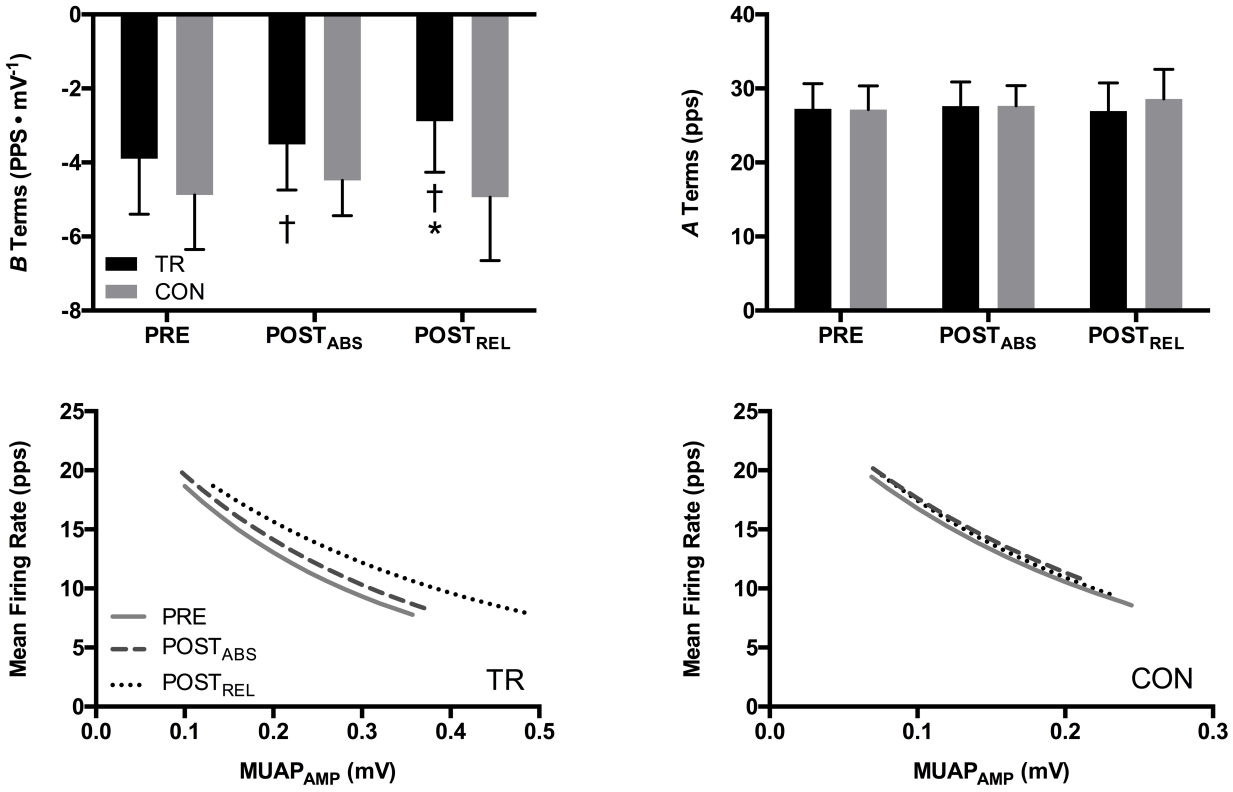
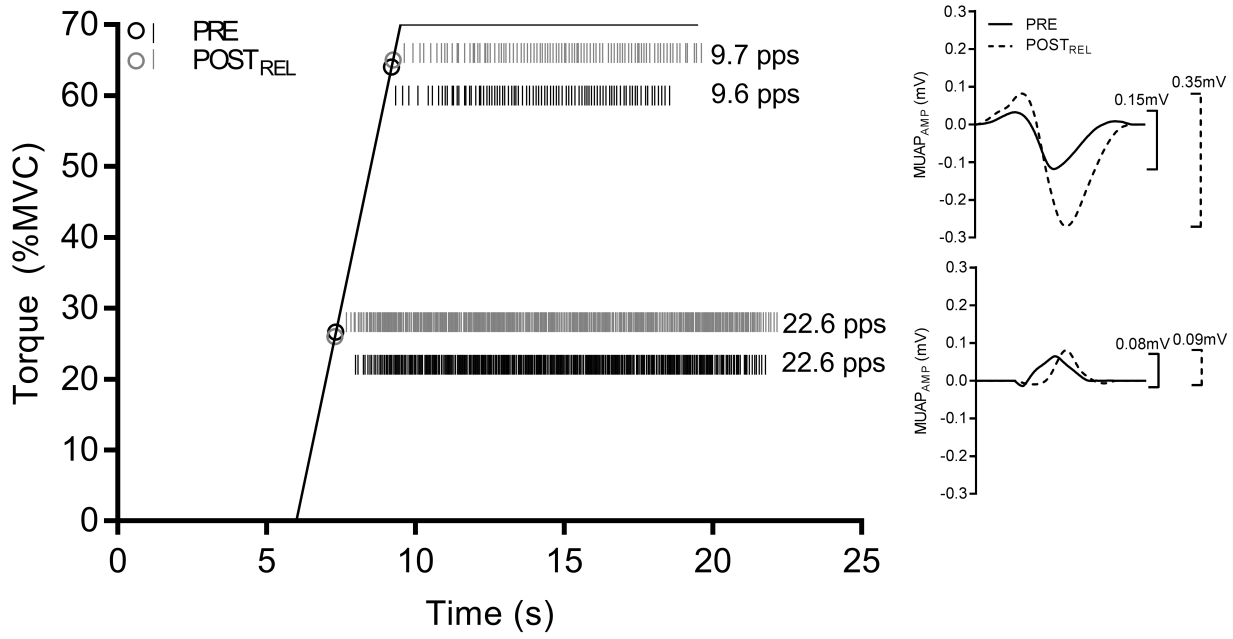


Figure 6. Left graph: Lower- and higher-threshold motor units (MUs) recruited at similar relative torques (expressed as percentage of maximal voluntary contraction [%MVC]) pre- (dark circles) and post-resistance training (gray circles) over laid on the linear segment of the isometric trapezoid template of the PRE and $POST_{REL}$ for one subject. The MU firing rates (vertical lines) were similar pre- and post-resistance training. Right graphs: The MU action potential amplitude ($MUAP_{AMP}$) was larger post-resistance training (dashed line) than pre-resistance training (solid line) for the higher-threshold MU, unlike for the lower-threshold MU.



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5. THE EFFECTS OF RESISTANCE TRAINING ON MOTOR UNIT FIRING RATES AND RECRUITMENT DURING REPETITIVE SUBMAXIMAL CONTRACTIONS

5.0 ABSTRACT

The purpose of this investigation was to examine the effects of an eight-week resistance-training program on the relationships between motor unit (MU) action potential amplitudes (AP_{AMPS}), mean firing rates (MFR) at steady torque, and recruitment thresholds (RT) of the vastus lateralis (VL) during repetitive submaximal contractions. Nineteen males (age: 20.6 ± 1.8 years; height: 179.0 ± 7.3 cm; weight: 75.7 ± 10.4 kg) completed an eight-week resistance-training program. Pre- and post-treatment, subjects performed to consecutive contractions at 40% of the pre-training MVC. $MUAP_{AMPS}$, MFRs, and RTs of the observed MUs were analyzed. Linear regression models were fitted to the $MUAP_{AMP}$ and MFR vs. RT relationships with the y-intercepts and slopes calculated. An exponential model fitted to the MFR vs. $MUAP_{AMP}$ relationships with the *A* (scale factor) and *B* (rate of decay) terms calculated. In addition, normalized EMG amplitude ($N\text{-}EMG_{RMS}$) and twitch torque potentiation were analyzed. Post-training, reduced muscle activation was observed in both contractions relative to pre-training as evidence by reduced $N\text{-}EMG_{RMS}$. Additionally, reduced MFR vs. RT and MUAP vs. RT slopes were observed in second post-training contraction relative to the first post-training contraction, indicating reduced MU recruitment to sustain the same torque. The findings suggest that subjects were able to produce both post-training contractions with reduced excitation to the motoneuron pool compared to pre-training. The reduced excitation in the second, relative to the first post-training contraction, suggests a resistance training induced increase in MU twitch potentiation, reducing the firing rates and recruitment necessary to sustained the desired torque.

5.1 INTRODUCTION

The force produced by a muscle is dictated by the magnitude of excitation to the motoneuron pool. As force demands increase, the greater excitation to the motoneuron pool simultaneously increases motor unit (MU) firing rates and the recruitment of larger MUs (Henneman's size principle) (1-6). In addition, the larger newly recruited MUs achieve firing rates lower than the earlier recruited smaller MUs, in accordance with the *onion skin* control scheme (3, 7-15). Though these underlying motor control schemes are unaffected, contractile activity can influence the excitation to the motoneuron pool required to produce a given force in subsequent contractions via fatigue or twitch potentiation.

MU twitch potentiation, attributed to the phosphorylation of myosin regulatory light chains by previous contractile activity, can temporarily increase MU twitch forces (16-18). As a result of the greater MU twitch forces, lower excitation to the motoneuron pool is required to produce the same force. As both MU firing rates and recruitment are dictated by the excitation to the motoneuron pool, the lower excitation reduces MU firing rates and recruitment required to produce a given force (19-22). Alternatively, previous contractions can diminish MU force twitches (fatigue) (23), thus requiring greater excitation to the motoneuron pool to achieve the same force (19). In contrast to twitch potentiation, fatigue has been reported to result in an increase MU firing rates and recruitment during subsequent contractions at the same force (9, 19, 24-27).

Resistance training may be able to influence the excitation required to match a given force in repeated contractions by increasing fatigue resistance and/or increasing MU twitch force potentiation as a result of the previous contraction. Resistance training has been shown to reduce

the number of total MUs necessary to produce a given force in cross-sectional and longitudinal investigations (28, 29). In a cross-sectional study, Sterczala et al (28) demonstrated that resistance trained men were able to produce 40% MVCs of the first dorsal interosseous with reduced MU recruitment in comparison to physically active controls. Similarly, several investigations have demonstrated that following a resistance training intervention, subjects were able to sustain the same absolute forces with lower muscle activation (29, 30). Additionally, resistance training may increase potentiation as observed in highly resistance trained individuals compared to sedentary controls (31). Therefore, resistance training can decrease MU recruitment at the same absolute force while also increasing MU twitch potentiation. Thus, resistance training may alter MU control strategies during a repetitive contractions, which has yet to be tested..

Analysis of MU firing rates, action potential (MUAP) amplitudes and recruitment thresholds may provide insight into the excitation to the motoneuron pool. Firing rates can be expressed relative to recruitment thresholds or MUAP amplitudes to detect changes in firing rates of the motoneuron pool. In additional, MUAP amplitudes can be expressed relative to recruitment thresholds to determine if the recruitment of larger MUs has been accelerated or delayed. These relationships have previously indicated that resistance training alters MU recruitment patterns, but not firing rates of motoneurons (28, 29).

The purpose of this investigation was to determine the effects of resistance training on MU recruitment and firing rate patterns of the vastus lateralis during repetitive submaximal contractions. Specifically, this investigation will determine if the relationships between firing rates at steady torque and recruitment thresholds, MUAP amplitudes and recruitment thresholds, and firing rates and MUAP amplitudes change as a result of diminished MU force twitches

(fatigue) pre-resistance training or increased MU twitch potentiation following an eight-week resistance training program. It is hypothesized that resistance training will increase MU twitch potentiation during the first contraction and, thus, lowering the neural excitation required to perform the second contraction. Lower neural excitation will lead to fewer recruited MUs and altered firing rates at steady torque.

5.2 METHODS

Subjects

Nineteen untrained, college-aged men (age: 20.6 ± 1.8 years; height: 179.0 ± 7.3 cm; weight: 75.7 ± 10.4 kg) completed this investigation. Subjects were physically active, none reported lower body resistance training in the previous six months. Additionally, none reported neuromuscular conditions or skeletal muscle injuries, which could influence the results of the investigation. The institutional review board for human subjects approved this investigation.

Experimental Approach

To determine if resistance training influenced MU recruitment and firing rates during consecutive contractions, subjects were tested before (PRE) and after (POST) an eight week, lower body resistance training program. Prior to each testing visit, subjects completed a familiarization visit, at which knee extension isometric maximal voluntary contractions (MVCs) and submaximal isometric trapezoid muscle actions were practiced. At each experimental visit, subjects performed two MVCs followed by two consecutive isometric trapezoidal muscle actions pre- (PRE 1 and 2) and post- resistance training (POST 1, and 2) at the same absolute torque.

The targeted steady torque was set at 40% MVC of the pre-resistance training strength. Surface electromyography (sEMG) signals collected from the vastus lateralis (VL) during all contractions provided a global measure of muscle activation. sEMG signals were decomposed to analyze MU firing rates, recruitment thresholds, and MUAP amplitudes. Electrical nerve stimulation was applied prior and after the first contraction at each visit to measure twitch torque potentiation.

Resistance Training Program

The resistance-training program included twenty-four total training sessions; three training sessions per week for eight weeks. At each visit, subjects performed four lower body exercises including back squats, front squats, Romanian deadlifts, leg extensions, leg presses, glute bridges, step ups, hamstring curls, and reverse hyperextensions. The resistance training program was based on a linear periodization model with volume decreasing and intensity increasing over the course of the program. Subjects performed three sets of twelve repetitions during weeks 1-3, three sets of eight repetitions during weeks 4-6 and four sets of five repetitions during weeks 7-8. The intensity for each set was based on the repetitions in reserve scale previously reported by Helms et al. (32). After each set the subjects reported their repetitions in reserve rating, and that rating along with the trainer's judgement was used to determine the intensity of the following set. National Strength and Conditioning Association Certified Strength and Conditioning Specialists oversaw all training sessions.

Isometric Strength Testing

Isometric strength testing of the right knee extensors was performed on a on a Biodex System 3 isometric dynamometer. Subjects were seated with restraining straps positioned over the pelvis, trunk and contralateral thigh. The right knee was positioned at 90° flexion with the lateral condyle of the femur aligned dynamometer's input axis of the dynamometer. Isometric strength was measured as the torque signal from the dynamometer.

The pre- and post-resistance training testing visits began with two three-second isometric MVCs of the right knee extensors. The highest 0.25s torque epoch observed from the pre-resistance training testing visit was used to determine the torque level for the submaximal contractions for pre- and post-resistance training. Following the MVCs, subjects performed two consecutive isometric trapezoidal muscle actions at 40% of the pre-resistance training MVC. Each isometric trapezoidal muscle consisted of a linear torque increase, a 40s plateau and a linear torque decrease. During muscle actions, torque increased and decreased at a rate of 20% MVC/s. Subjects were provided with real-time torque feedback and instructed to trace a visual template.

Electromyography

sEMG signals were collected from the VL via a Delsys 5-pin surface sensor array consisting of five 0.5 mm pins arranged in a 5x5 mm square with the fifth pin positioned in the center. The sensor was positioned at approximately 60% of the distance between the greater trochanter and the lateral condyle of the femur and a reference electrode was placed over the left patella. Prior to the sensor and reference electrode placement, the sites were shaved, dry skin cells were removed via repeated application of adhesive tape, and the sites was sterilized with

alcohol. The signals from the electrode's four pairs of the pins were differentially amplified and filtered at a band-pass of 20-450 Hz. The EMG signals were sampled at 20 kHz and stored for subsequent decomposition and analysis. The four channels of surface EMG data collected by the 5-pin sensor array were decomposed into their constituent MU action potential trains using the Precision III algorithm. The accuracy of the decomposed action potential trains were tested via the reconstruct-and-test procedure. Only MUs that demonstrated ≥ 90% accuracy were included in the subsequent analyses (33). In addition, MUs recruited or derecruited during the steady force region of the trapezoidal muscle actions were excluded from the analysis.

The action potential trains were low-pass filtered with a unit area Hanning window (2s duration) to compute the firing rates of each MU. Analyses yielded four parameters per MU: the recruitment threshold (RT), mean firing rate (MFR), MUAP amplitude ($MUAP_{AMP}$) and the MUAP duration ($MUAP_{DUR}$). The RT was calculated as the average torque during the 0.01 second epoch following the first firing of the MU and was expressed relative to the MVC torque. The MFR was calculated as the average firing rate during the entire steady torque region of the isometric trapezoidal muscle action. The $MUAP_{AMP}$ was calculated as the average peak-to-peak amplitude of the each of the four unique action potential waveform templates in accordance with previously reported methods (3, 5, 28). The $MUAP_{DUR}$ was calculated as the average peak-to-peak duration of the four unique action potential waveform templates.

The EMG signal from the first of the four channels of the surface sensor array was used to measure gross muscle activation in the VL. The EMG signals were bandpass filtered (zero phase fourth-order Butterworth filter) at 10-500 Hz. The average EMG root mean square (EMG_{RMS}) during the steady torque region of the isometric trapezoidal muscle actions was

normalized (N- EMG_{RMS}) to the EMG_{RMS} of the highest 0.25 s torque (Peak TQ) epoch observed during the respective visits MVCs.

Twitch Torque Potentiation

Non-voluntary twitch torques were evoked using transcutaneous electrical stimuli delivered to the femoral nerve via a high-voltage, constant-current stimulator (Digitimer DS7AH, Herthfordshire, UK). The optimal stimulus location was determined by administering relatively low current single stimuli (amperage = 80 mA) via a bipolar probe. After identifying the optimal location, further stimuli were administered via adhesive electrodes. To determine the amperage necessary to elicit a maximal contraction, stimuli were delivered beginning at 80 mA and increasing 10 – 20 mA until the elicited twitch torque plateaued for three straight stimuli. The supramaximal stimulus used to evoke twitches was 120% of the third amperage to evoke the maximal twitch torque. The twitches were evoked immediately before and after the first contraction, respectively. Peak twitch torques were calculated as the average of the highest 0.05s of torque. Twitch torque potentiation (TQ_{POT}) was calculated as the peak twitch torque prior to the contraction minus the peak twitch torque following the contraction.

Subcutaneous Fat Measurement

To ensure that changes in subcutaneous fat thickness did not influence the sEMG data, transverse panoramic images of the right VL were captured using brightness mode ultrasound imaging. Images were captured using a NextGen LOGIQ c ultrasound console (GE Healthcare UK, Ltd., Chalfont, Buckinghamshire, UK) with a multi-frequency linear array transducer

(Model 12L-RS; 5-13 MHz; 38.4 mm field-of-view). Equipment settings were kept constant across all subjects (depth: 6.0 cm; gain: 49 dB; frequency: 10 MHz). Prior to imaging, subjects rested supine for 10 minutes to allow for fluid shifts. Images were collected at 50% of the distance from the anterior superior iliac spine to the superior border of the patella. Images were analyzed using the straight-line function in ImageJ software. Subcutaneous fat was measured as the straight-line distance from the bottom of the cutaneous layer to the top of the muscle fascia.

Statistical Analysis

For MU analysis, the MFR vs. RT, $MUAP_{AMP}$ vs. RT, and MFR vs. $MUAP_{AMP}$ relationships for each subject's contraction were calculated. MFRs and $MUAP_{AMPS}$ were linearly regressed against RT to yield slope and y intercept values. An exponential model was applied to MFR vs. $MUAP_{AMP}$ relationships to yield *B* and *A* terms. In this model, $MFR = Ae^{B(MUAPAMP)}$, where *A* is the MFR scale factor, *e* is the natural constant, and *B* is the rate of MFR decay with increases in $MUAP_{AMP}$. These relationships can be biased by the RT range and RT distribution of the recorded MUs. Subjects with contractions that had a RT range (maximum RT – minimum RT) less than 20% MVC were excluded from analysis. In addition, several subjects met the RT range criteria, however, nearly all recorded MUs had RTs near the steady torque (between 35 – 40% MVC) and resulted in spurious regression coefficients . Therefore, subjects that did not have at least 10 recorded MUs with RTs below 35% MVC were also excluded from analysis.

$MUAP_{DURS}$ were calculated as an indirect measure of MU depth. Only one significant $MUAP_{DUR}$ vs. RT relationship was observed in the thirty-six contractions ($r = 0.264 \pm 0.196$). The lack of significant relationships between $MUAP_{DUR}$ and RT suggest MUs of similar depth

were recorded across the RT spectrum. Therefore, interpretation of MFR and $MUAP_{AMPS}$ in relation to RT were not influenced by the depth of recorded MUs. Due to the lack of significant relationships, the average $MUAP_{DUR}$ for each subject's contraction was statistically analyzed.

The slopes and y-intercepts of the MFR vs. RT and $MUAP_{AMP}$ vs. RT relationships, *B* and *A* terms of the MFR vs. MUAP relationships, average $MUAP_{DURS}$ and $N\text{-}EMG_{RMS}$ were analyzed via eight separate two-way repeated measures ANOVAs [Time (PRE vs. POST) x Repetition (1 vs. 2)]. When appropriate, follow-up analyses were performed using independent samples t-tests. Potential changes in Peak TQ, TQ_{POT}, and sFAT were examined via independent samples t-tests. All statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, NY) with alpha was set at $p \leq 0.05$.

5.3 RESULTS

MU Data

Of the nineteen subjects, nine met the RT range and distribution criteria for all four (2 PRE, 2 POST) 40% MVCs. sEMG signal decomposition from these nine subjects yielded 1,367 total MUs across the two pre-resistance training contractions and two post-training 40% MVCs. Similar average MU counts were observed in each of the four contractions (PRE 1: 34.7 ± 7.0; PRE 2: 37.8 ± 6.6; POST 1: 38.9 ± 10.5; POST 2: 40.6 ± 11.2). The RT ranges of the recorded MUs were similar for all four contractions (PRE 1: 32.7 ± 6.6% MVC; PRE 2: 34.2 ± 3.5% MVC; POST 1: 33.8 ± 4.9% MVC; POST 2: 37.0 ± 5.9% MVC). Significant correlations were observed for all linear MFR vs. RT (r = -0.955 ± 0.347), linear $MUAP_{AMP}$ vs. RT (r = 0.803 ± 0.080), and exponential MFR vs. $MUAP_{AMP}$ relationships (r = -0.820 ± 0.082).

MFR vs. RT Relationships

For the slopes, there was a significant time x repetition interaction ($p = 0.043$). Paired samples t-tests indicated that there were not differences between the slopes for PRE 1 and PRE 2 ($p = 0.109$). POST 2, however, was significantly less negative than POST 1 ($p < 0.001$) (Figure 1). There were no significant differences between the PRE 1 and POST 1 ($p = 0.368$) and PRE 2 and POST 2 ($p = 0.114$). For the y-intercepts, there were no significant time x repetition interaction ($p = 0.414$) nor a significant main effect for repetition ($p = 0.051$). There was a significant main effect for time with greater y-intercepts observed post- in comparison to pre-resistance training ($p = 0.037$).

$MUAP_{AMP}$ vs. RT Relationships

For the slopes, there was a significant time x repetition interaction ($p = 0.020$). Slopes were not significantly different between PRE 1 and PRE 2 ($p = 0.134$), however, POST 2 had smaller slopes compared to POST 1 ($p = 0.025$) (Figure 2). There were no significant differences between PRE 1 and POST 1 ($p = 0.142$) or POST 1 and POST 2 ($p = 0.301$). For the y-intercepts, there was no significant time x repetition interaction ($p = 0.142$), main effect for time ($p = 0.081$) or main effect for repetition ($p = 0.795$).

For the MFR vs. $MUAP_{AMP}$ Relationships

For the *B* terms, there were no significant time x repetition interaction ($p = 0.178$) or main effect for time ($p = 0.208$), however, there was a significant main effect for repetition ($p =$

0.036). The *B* terms of the second 40% MVCs (PRE 2 and POST 2) were more negative than the first 40% MVCs (PRE 1 and PRE 2) (Figure 3). For the *A* terms, there was no significant time x repetition interaction (p = 0.091) or main effects for time (p = 0.121) or repetition (p = 0.639).

$MUAP_{DURS}$

MUs of similar depths were recorded in all four contractions as evidenced by the lack of significant time x repetition interaction (p = 0.244) or main effects for time (p = 0.993) and repetition (p = 0.311). The observed average $MUAP_{DURS}$ for PRE 1, PRE 2, POST 1, and POST 2 were 5.59 ± 0.43 ms, 5.59 ± 0.20 ms, 5.67 ± 0.39 ms, and 5.51 ± 0.36 ms, respectively.

$N\text{-}EMG_{RMS}$

There was no significant time x repetition interaction (p = 0.887) or main effect for repetition (p = 0.196). There was a significant main effect for time (p = 0.001) indicating significantly lower $N\text{-}EMG_{RMS}$ at post-resistance training (22.63 ± 7.21%) compared to pre-resistance training (32.19 ± 8.59%) when collapsed across repetitions.

Peak TQ, TQ_{POT}, and sFAT

The resistance training program significantly increased Peak TQ (p = 0.003; PRE: 188.5 ± 27.6 Nm; POST: 223.2 ± 38.6 Nm). There was no significance difference in TQ_{POT} pre- to post-resistance training (p = 0.535; Pre: 2.63 ± 4.28 Nm; Post: 3.48 ± 3.59 Nm). The resistance

training program had no effect on sFAT ($p = 0.757$); PRE: 0.57 ± 0.27 cm; POST: 0.56 ± 0.23 cm).

5.4 DISCUSSION

In this investigation, untrained individuals demonstrated similar MU recruitment and firing rate patterns during two consecutive submaximal contractions as measured by the MFR vs. RT, $MUAP_{AMP}$ vs. RT, and MFR vs. $MUAP_{AMP}$ relationships. Following eight weeks of resistance training, significant differences in these relationships were observed between the two contractions. Notably, post-resistance training, the slopes of the MFR vs. RT relationship were less negative and the slopes of the $MUAP_{AMP}$ vs. RT relationship were smaller. These attenuated slopes may indicate reduced recruitment during the second contraction, possibly due to potentiation of the MUs recruited in the first contraction. In addition, the reduced $N\text{-}EMG_{RMS}$ suggests that the resistance training reduced the muscle activation required to perform the same force, even before the effects of MU twitch potentiation.

Neither the resistance training program nor the repetitive contractions affected the underlying motor control schemes. Pre- and post-resistance training, strong negative MFR vs. RT and $MUAP_{AMP}$ relationships were observed in all contractions indicating that later recruited and larger MUs possessed lower firing rates throughout the contractions than the earlier recruited and smaller MUs, in accordance with the onion skin firing scheme (3, 7). As $MUAP_{AMP}$ is correlated with the size the motoneuron (34) and the diameter of the MU's muscle fibers (35), the strong positive relationships between $MUAP_{AMP}$ and RT indicated orderly recruitment of MUs according to size in accordance with the size principle (3, 5, 34). These findings are in

agreement with previous investigations reporting that neither chronic activity, such as training (10, 28, 29, 36), nor acute activity, such as fatigue (24), affects the motor control schemes.

Prior to the resistance training program, both contractions demonstrated similar slopes and y-intercepts of the $MUAP_{AMP}$ vs. RT relationships. Thus, MUs with similar $MUAP_{AMPs}$ were recruited at the same RT in both contractions. Post-resistance training, similar y-intercepts were observed in both contractions, however, significantly slower slopes were observed in POST 2 compared to POST 1. As $MUAP_{AMP}$ provides an indirect measure of MU size (3, 5, 34, 35), the decrease in slopes suggest a rate of rise in MU size with increases in RT. As a result, MUs of a similar size were recruited at higher RTs in POST 2 (Figure 2). Given that MUs are recruited in an orderly fashion according to size (1), these findings also suggest the same absolute force in the second contraction was achieved with fewer recruited MUs. Fewer recruited MUs to maintain the steady force during the second 40% MVC was likely the result of increased MU twitch force potentiation. The reduced MU recruitment was not due to fatigue, as fatigue transiently reduces MU RTs (9, 24), resulting in the recruitment of MUs with larger $MUAP_{AMPS}$ (24, 37). Instead, the attenuated slopes indicate reduced excitation to the motor unit pool, likely due to MU potentiation (19-22).

The MFR vs. RT relationships paralleled the findings from the $MUAP_{AMP}$ vs. RT relationships. Pre-resistance training, no differences were observed for the slopes or y-intercepts from the MFR vs. RT relationships between PRE 1 and PRE 2, indicating similar firing rate behavior in both contractions. Post-resistance training, no differences were observed in the y-intercepts of the MFR vs. RT relationships suggesting minimal or no alteration in the firing rates of the lowest-threshold MUs. Although firing rates of lower-threshold MU were unaltered, significantly less negative slopes from the relationships were observed in POST 2 compared to

POST 1 indicating a smaller decline in MFR with increases in RT. Alone, the attenuated slopes could indicate a firing rate increase in the higher threshold MUs as has been observed in fatigued conditions (19, 24-27). Considering no changes in the $MUAP_{AMP}$ vs. RT relationships, these findings suggest that the alteration in the MFR vs. RT relationships was due to reduced MU recruitment. As a result of the reduced recruitment of larger MUs, smaller MUs were recruited at higher RTs during the second repetition and, thus, increasing the MFR vs. RT relationship slope (Figure 1).

The MFR vs. $MUAP_{AMP}$ relationships provide further evidence that the attenuated MFR vs. RT and $MUAP_{AMP}$ vs. RT relationships observed in POST 2 were due to MU twitch potentiation induced by POST 1. Statistical analyses indicated a significant main effect for time, suggesting that for pre- and post-resistance training, *B* terms of the MFR vs. $MUAP_{AMP}$ relationships were more negative in POST 2. Further analysis, however, suggests that this main effect for time was due to post-training contractions, as only a very small effect size was observed for the pre-training contractions (PRE1: -4.413 ± 0.683; PRE2: -4.425 ± 0.887; cohens d: 0.015), but a large effect size was observed for the post-resistance training contractions (POST1: -3.796 ± 0.709; POST2: -4.433 ± 0.733; cohen's d: 0.824). The more negative *B* terms observed post-resistance training indicate that the smallest MUs were firing at similar rates in both contractions, however, the larger active MUs were firing slower. This decrease in the firing rates of the larger, later recruited MUs is consistent with reduced excitation to the motoneuron pool. Given the sigmoidal relationship between MU firing rates and excitation, the MU twitch potentiation-related reduction in required excitation would have little impact on the smaller, earlier recruited MUs. In contrast, the largest, last recruited MUs would experience greater decreases in firing rates.

The lower N-EMG_{RMS} (PRE: 32.19 ± 8.59; POST: 22.63 ± 7.21) observed post-resistance training suggests reduced muscle activation to sustain the same absolute force, potentially due to greater MU twitch forces. Greater MU twitch forces would reduce the number of total MUs and firing rates required to maintain the same absolute force (19-22)and, thus, lowering muscle activation. Although individual MU twitch forces were not measured in the present investigation, support for an increased MU twitch forces is provided by the increase in maximal leg extension torque. Despite the differences in the MFR vs. RT and $MUAP_{AMP}$ vs. RT relationships, no difference in N-EMG_{RMS} was observed between POST1 and POST 2. The lack of change in N-EMG_{RMS} between POST 1 and POST 2 is not surprising, however, as N-EMG_{RMS} only provides a crude measure of muscle activation and may not be sensitive to small changes in MU behavior (38).

Although the alterations in MU relationships indicate greater potentiation, no increase in TQ_{POT} was observed post-resistance training. As the first 40% MVC served as the conditioning contraction, only the MUs recruited during the first contraction would be potentiated, whereas, MU twitch potentiation was measured using evoked twitches, which elicited complete MU recruitment. The evoked twitches were more representative of the largest, strongest high-threshold MUs that would not have been recruited during the 40% MVCs. Additionally, the lack of difference in TQ_{POT}, may be due to the greater muscle activation observed pre-training. As more MUs were recruited pre- than post-resistance training, a greater number of MUs would have been potentiated. Thus, increased TQ_{POT} of individual MUs may have been masked by the greater MU recruitment pre-resistance training.

A limitation of the present study is that MU behavior was only measured in the vastus lateralis, and not the synergist or antagonist muscles. Previously, Contessa et al. (25) reported

that alterations of the MFR vs. $MUAP_{AMP}$ relationship during fatiguing contractions were associated with increased co-activation (25). The altered MFR vs. RT, $MUAP_{AMP}$ vs. RT and MFR vs. $MUAP_{AMP}$ relationships observed in the present study are not likely due to co-activation, however, as no change in agonist muscle $N\text{-}EMG_{RMS}$ was observed, whereas, Contessa et al. (25) observed a decrease in agonist $N\text{-}EMG_{RMS}$ concurrent with the increases in muscle co-activation.

In summary, MU behavior during repeated contractions was significantly altered following an eight-week resistance training program. No differences in MFR vs. RT, $MUAP_{AMP}$ vs. RT and MFR vs. $MUAP_{AMP}$ relationships were observed between the two pre-training contractions. In contrast, significantly attenuated slopes from the MFR vs. RT and $MUAP_{AMP}$ vs. RT relationships were observed for POST 2 compared to POST 1, indicating fewer recruited MUs. Additionally, the slopes from the MFR vs. $MUAP_{AMP}$ relationship suggest that the larger MUs were firing at slower rates in POST 2 compared to POST 1. Together, these findings suggest that resistance training induced increases in MU twitch potentiation that resulted in lower overall neural excitation to perform the second contraction. Thus, resistance training can significantly reduce fatigue in repetitive actions due to potentiation induced reductions in required muscle activation.

5.5 FIGURES

Figure 1. The mean (SD) slopes of the firing rate vs. recruitment threshold (expressed as percent of maximal voluntary contraction [%MVC]) relationship (top), mean (SD) y-intercepts (middle), and composite pattern for pre- (PRE 1, PRE 2) and post-training repetitive (POST 1, POST 2) contractions. * Indicates that POST 2 was significantly different from POST 1. † Indicates that the post-training contractions were significantly different from the pre-training contractions.

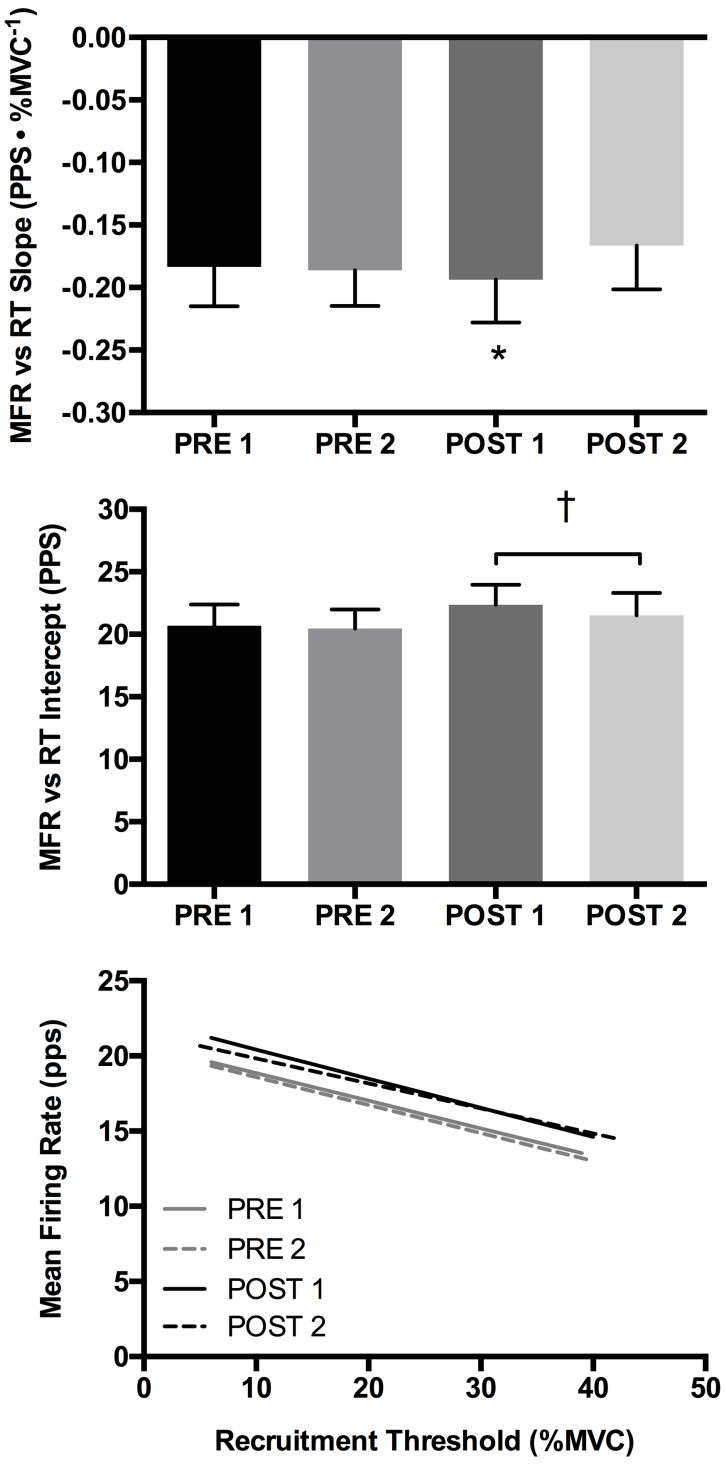


Figure 2. The mean (SD) slopes of the motor unit action potential amplitude ($MUAP_{AMP}$) vs. recruitment threshold (expressed as percent of maximal voluntary contraction [%MVC]) relationship (top), mean (SD) y-intercepts (middle), and composite pattern for pre- (PRE 1, PRE 2) and post-training repetitive (POST 1, POST 2) contractions. * Indicates that POST 2 was significantly different from POST 1.

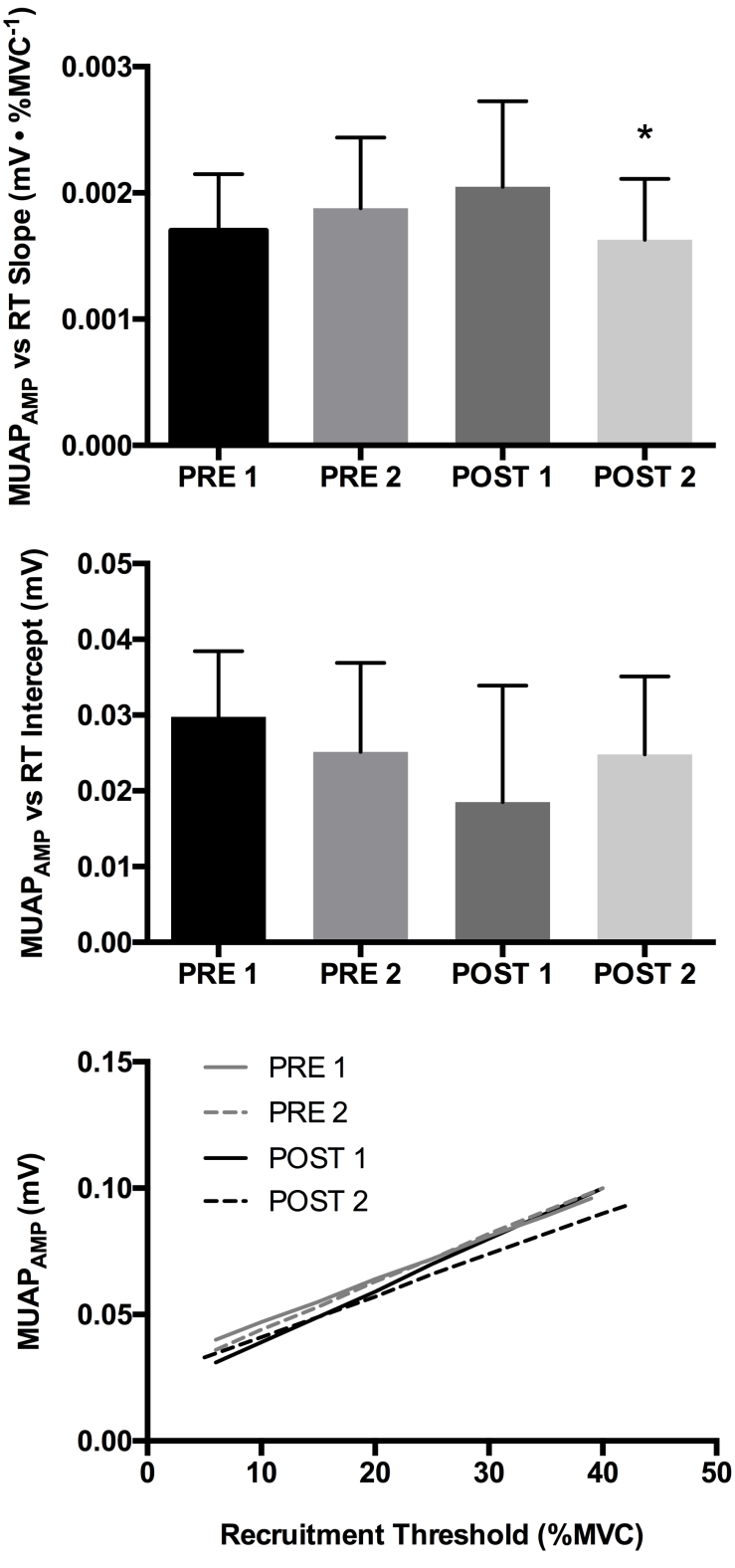
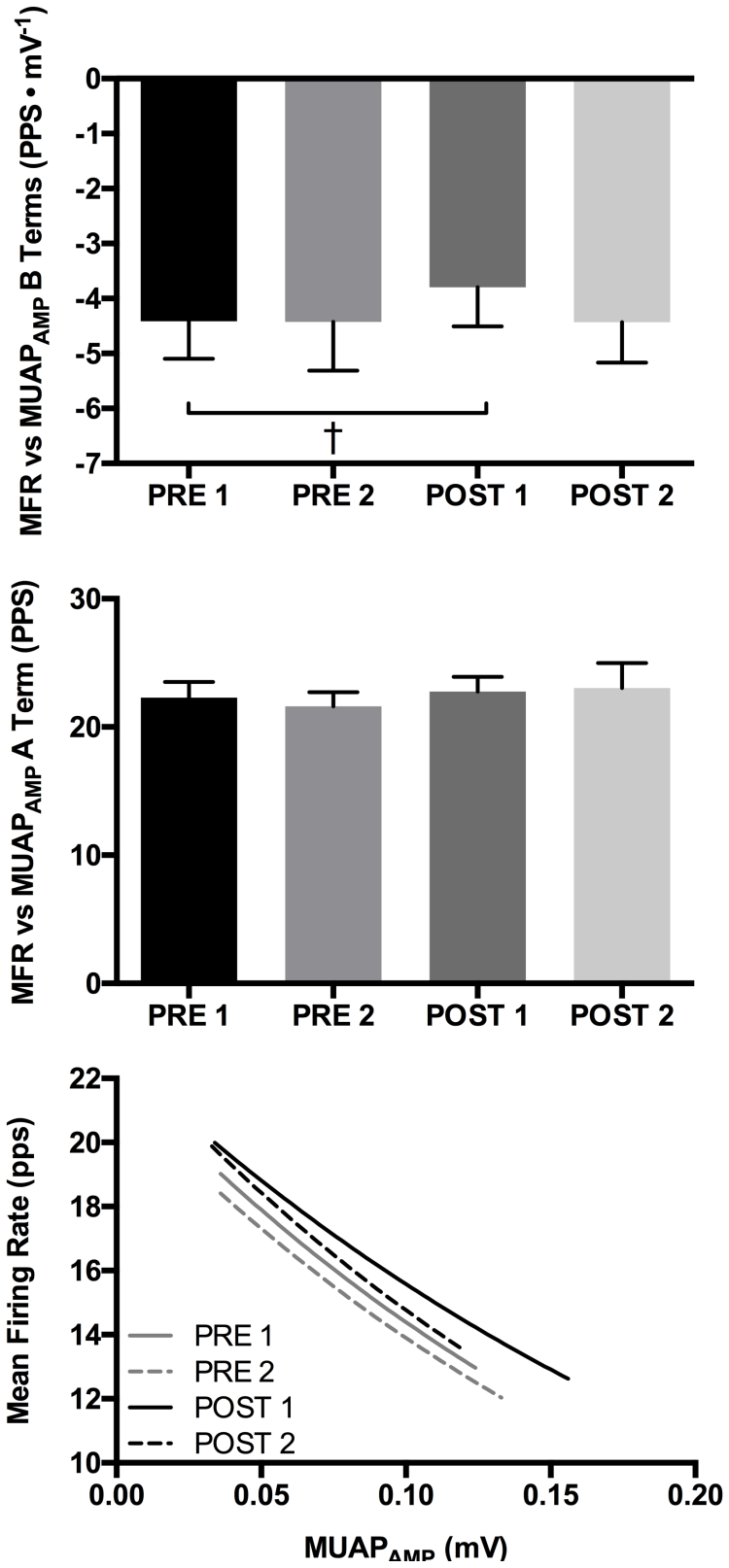


Figure 3. The mean (SD) *B* terms of the firing rate vs. motor unit action potential amplitude ($MUAP_{AMP}$) relationship (top), mean (SD) *A* terms (middle), and composite pattern for pre- (PRE 1, PRE 2) and post-training repetitive (POST 1, POST 2) contractions. † Indicates that the first contractions at each time point (PRE 1 & PRE 2) were significantly different from the second contractions (PRE 2 & POST 2) at each time point.



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